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1

CELL CYCLE PROGRESSION PROTEINS

The present invention relates to a number of genes implicated in the processes of cell cycle progression, including mitosis and meiosis.

We have now identified a large number of genes in *Drosophila*, mutations in which disrupt cell cycle progression, for example the processes of mitosis and/or meiosis. We have determined the phenotypes of these mutations and recovered nucleotide sequences associated with the corresponding genes. Many of these nucleotide sequences correspond to protein open reading frames (ORFs) present in the *Drosophila* genome.

Accordingly the present invention provides in one aspect a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 70 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 70, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 70 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

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There is provided, according to another aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 14 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 14, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 14 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

We provide, according to yet a further aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide

sequences set out in Examples 15 to 19 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 15 to 19, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 15 to 19 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

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As a further aspect of the present invention, there is provided a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 20 to 30 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 20 to 30, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 20 to 30 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

We provide, according to a yet further aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 31 to 53 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 31 to 53, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 31 to 53 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

The present invention, in a further aspect, provides a polynucleotide selected from:
(a) polynucleotides comprising any one of the nucleotide sequences set out in 54 to 70 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in 54 to 70, or a fragment thereof; (c)

WO 01/72774

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PCT/GB01/01297

3

polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 54 to 70 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a 5 polynucleotide according to any of the above aspects of the invention.

The present invention also provides a polypeptide which comprises any one of the amino acid sequences set out in Examples 1 to 70 or in any of Examples 1 to 14, Examples 15 to 19, Examples 20 to 30, Examples 31 to 53 and Examples 54 to 70, or a homologue, variant, derivative or fragment thereof.

Preferably the polypeptide is encoded by a cDNA sequence obtainable from a eukaryotic cDNA library, preferably a metazoan cDNA library (such as insect or mammalian) said DNA sequence comprising a DNA sequence being selectively detectable with a Drosophila nucleotide sequence as shown in any one of Examples 1 to 70.

The term "selectively detectable" means that the cDNA used as a probe is used under conditions where a target cDNA of the invention is found to hybridize to the probe at a level significantly above background. The background hybridization may occur because of other cDNAs present in the cDNA library. In this event background implies a level of signal generated by interaction between the probe and a non-specific cDNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target cDNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ³²P. Suitable conditions may be found by reference to the Examples, as well as in the detailed description below.

A polynucleotide encoding a polypeptide of the invention is also provided.

The present invention further provides a vector comprising a polynucleotide of the invention, for example an expression vector comprising a polynucleotide of the invention

4

operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.

Also provided is an antibody capable of binding a polypeptide of the invention.

In a further aspect the present invention provides a method for detecting the presence or absence of a polynucleotide of the invention in a biological sample which method comprises: (a) bringing the biological sample containing DNA or RNA into contact with a probe comprising a nucleotide of the invention under hybridising conditions; and (b) detecting any duplex formed between the probe and nucleic acid in the sample.

In another aspect the invention provides a method for detecting a polypeptide of the invention present in a biological sample which comprises: (a) providing an antibody of the invention; (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said antibody is formed.

Knowledge of the genes involved in cell cycle progression allows the development of therapeutic agents for the treatment of medical conditions associated with aberrant cell cycle progression. Accordingly, the present invention provides a polynucleotide of the invention for use in therapy. The present invention also provides a polypeptide of the invention for use in therapy. The present invention further provides an antibody of the invention for use in therapy.

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In a specific embodiment, the present invention provides a method of treating a tumour or a patient suffering from a proliferative disease, comprising administering to a patient in need of treatment an effective amount of a polynucleotide, polypeptide and/or antibody of the invention.

The present invention also provides the use of a polypeptide of the invention in a method of identifying a substance capable of affecting the function of the corresponding

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PCT/GB01/01297

gene. For example, in one embodiment the present invention provides the use of a polypeptide of the invention in an assay for identifying a substance capable of inhibiting cell cycle progression. The substance may inhibit any of the steps or stages in the cell cycle, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, and cytokinesis functions. For example, possible functions of genes of the invention for which it may be desired to identify substances which affect such functions include chromatin binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

In a further aspect the present invention provides a method for identifying a substance capable of binding to a polypeptide of the invention, which method comprises incubating the polypeptide with a candidate substance under suitable conditions and determining whether the substance binds to the polypeptide.

In an additional aspect, the invention provides kits comprising polynucleotides, polypeptides or antibodies of the invention and methods of using such kits in diagnosing the presence of absence of polynucleotides and polypeptides of the invention including deleterious mutant forms.

Also provided is a substance identified by the above methods of the invention.

Such substances may be used in a method of therapy, such as in a method of affecting cell cycle progression, for example mitosis and/or meiosis.

6

The invention also provides a process comprising the steps of: (a) performing one of the above methods; and (b) preparing a quantity of those one or more substances identified as being capable of binding to a polypeptide of the invention.

Also provided is a process comprising the steps of: (a) performing one of the above methods; and (b) preparing a pharmaceutical composition comprising one or more substances identified as being capable of binding to a polypeptide of the invention.

We further provide a method for identifying a substance capable of modulating the function of a polypeptide of the invention or a polypeptide encoded by a polynucleotide of the invention, the method comprising the steps of: incubating the polypeptide with a candidate substance and determining whether activity of the polypeptide is thereby modulated.

A substance identified by a method or assay according to any of the above methods or processes is also provided, as is the use of such a substance in a method of inhibiting the function of a polypeptide. Use of such a substance in a method of regulating a cell division cycle function is also provided.

DETAILED DESCRIPTION OF THE INVENTION

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The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; and, D.

M. J. Lilley and J. E. Dahlberg, 1992, Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA Methods in Enzymology, Academic Press. Each of these general texts is herein incorporated by reference.

Preferably, the polypeptides and polynucleotides of the invention are such that they
give rise to or are associated with defined phenotypes when mutated.

For example, mutations in the polypeptides and polynucleotides of the invention may be associated with a failure to complete cytokinesis; such polypeptides and polynucleotides are conveniently categorised as "Category 1". Phenotypes associated with Category 1 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: cytokinesis defect (polyploidy); Male 10 semi-sterile, Meiotic defects in testis: cytokinesis defects, segregation defects.(Seg-01/62); Meiotic defects in testis: cytokinesis defects, abnormal spindles.(Ab-02/12); Mitotic defects in brain: cytokinesis defect (no overcondensation of diploids, high polyploidy); Meiotic defects in testis: cytokinesis defects. Dark bands in eyes, dominant; Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis:segregation defect, 15 cytokinesis defect(Ck-09/32); Mitotic defects in brain: cytokinesis defect (no overcondensation of diploids, very high polyploidy); Mitotic defects in brain: cytokinesis defect(very high polyploidy); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defects (Mitotic higher level of condensation, polyploidy, Meiotic: Ck05/07); Mitotic defects in brain, Cytokinesis defect (no overcondensation of diploids, 20 high polyploidy); Mitotic defects in brain: cytokinesis defect (very high polyploidy, chromosomes entangled?); Mitotic defects in brain: cytokinesis defect (very high polyploidy; Meiotic defects in testis: cytokinesis defects (Ck-04/06) '; Female sterile (anaphase bridges, lagging chromosomes); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defects:(mitotic: high polyploidy, no diploids, higher 25 mitotic index, meiotic: Ck-01/05); Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis: cytokinesis defects(Ck-06/09); Meiotic defects in testis: segregation defects, cytokinesis defect(Ck-07/35); Meiotic defects in testis: cytokinesis defects.

8

Alternatively, mutations in the polypeptides and polynucleotides of the invention may be associated with a failure to enter M-phase; such polypeptides and polynucleotides are conveniently categorised as "Category 2". Phenotypes associated with Category 2 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Meiotic defects in testis: no division(no meiosis); Mitotic defects in brain: no mitosis; Meiotic defects in testis: segregation defects, meiotic failure(Mf-07/75); Meiotic defects in testis: segregation defects, meiotic failure(Mf-05/31); Meiotic defects in testis: cytokinesis defects, meiotic failure(Mf-02/15).

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Mutations in the polypeptides and polynucleotides of the invention may be associated with a metaphase arrest phenotype ("Category 3"). Phenotypes associated with Category 3 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: prometaphase arrest (overcondensation, polyploidy, scattered chromosomes with bipolar spindle); Male sterile, Female sterile, Mitotic defects in brain: prometaphase arrest (Overcondensation, polyploidy, fewer anaphases, high mitotic index, scattered chromosomes with bipolar spindle); Mitotic defects in brain: (weak overcondensation, metaphase with bipolar spindle); Mitotic defects in brain: prometaphase arrest; Mitotic defects in brain: metaphase arrest; Mitotic defects in brain: metaphase arrest. (overcondensation, polyploidy, aneuploidy, few anaphases, high mitotic index, metaphase with bent bipolar spindle); Mitotic defects in brain: metaphase arrest. (overcondensation, polyploidy, few anaphases, high mitotic index, metaphase with bent bipolar spindle); Mitotic defects in brain: Metaphase arrest (overcondensation, polyploidy, aneuploidy, no anaphases, high mitotic index, metaphase with bipolar spindle); Mitotic defects in brain: metaphase arrest (overcondensation, metaphase with bipolar spindle; Meiotic defects in testis: segregation defects, multipolar spindles (Mul-02/29); Meiotic defects in testis: cytokinesis defects, abnormal spindles. (Ab-01/03); Mitotic defects in brain: metaphase arrest; Mitotic defects in brain: metaphase arrest (overcondensation, polyploidy, metaphase with bipolar spindle); Mitotic defects in brain: metaphase arrest. Meiotic defects in testis: segregation defects. Abnormal spindles (mitotic: High mitotic index, meiotic: Ab-08/24); Mitotic defects in brain: metaphase arrest(overcondensation, few anaphases, some polyploids); Mitotic defects in brain: prometaphase arrest (overcondensation, fewer anaphases, metaphase with bipolar spindle);

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Mitotic defects in brain: metaphase arrest(condensation, no polyploidy, no anaphases, metaphase with bipolar spindle).

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Mutations in Category 4 polypeptides and polynucleotides of the invention may be associated with an anaphase defect phenotype; phenotypes associated with Category 4 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: anaphase defects (overcondensation, high polyploidy, some lagging chromosomes); Meiotic defects in testis: segregation defects; Male and female sterile, small wings, meiotic defects in testis: segregation defects, elongation defect; Mitotic defects in brain: anaphase defects(overcondensation, anaphase bridge, metaphase with swollen chromosomes and bipolar spindle); Mitotic defects in brain: Anaphase defects. (overcondensation, aneuploidy, some lagging chromosomes and breaks); Meiotic defects in testis: segregation defects; Meiotic defects in testis: segregation defects, multi-stage defects (Pl-02/17); Meiotic defects in testis: segregation defects, multi-stage defects (P1-02/18); Meiotic defects in testis: cytokinesis defects, segregation defects (seg-01/01); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defect. Multi-stage defects Polyploidy, no overcondensation PI-01/10; Meiotic defects in testis: segregation defects, abnormal spindles. (Ab-03/30); Mitotic defects in brain: anaphase defects (weak, higher condensation, some polyploidy, fewer anaphases, polyploids with monopolar spindles); Mitotic defects in brain: anaphase defects (overcondensation, polyplody (with overcondensation), few anaphases, metaphase with bipolar spindle); Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis: segregation defects, multipolar spindles (Mul-02/22); Meiotic defects in testis: segregation defects, abnormal spindles (Ab-04/26); Meiotic defects in testis: cytokinesis defects, abnormal spindles (Ab-16/13); Mitotic defects in brain: anaphase defects. Meiotic defects in testis: segregation defects, abnormal spindles (mitotic: Overcondensation, lagging chromosomes/less aligned metaphase with bipolar spindles, Meiotic: Ab-06/20); Meiotic defects in testis: segregation defects; Meiotic defects in testis: no division (no meiosis); Meiotic defects in testis: segregation defects, abnormal spindles (Ab-12/48); Meiotic defects in testis: segregation defects, multipolar spindles(mitotic: High polyploids, no diploids, higher mitotic index Meiotic: Mul-02/59); Meiotic defects in testis: segregation defect; Mejotic defects in testis: segregation defects, abnormal spindles

(meiotic: Ab-08/42); Female sterile. Meiotic defects in testis: cytokinesis defects, segregation defects (Mitotic: Less condensed chromosomes, nuclear bridges, Meiotic: Seg-01/02; Mitotic defects in brain: anaphase defects; Meiotic defects in testis: cytokinesis defects, abnormal spindles(Ab-01/04); Meiotic defects in testis: segregation defects(overcondensation, fewer anaphases); Mitotic defects in brain: (some overcondensation, anaphase bridge, metaphase with swollen chromosome and bipolar spindle).

A fifth category ("Category 5") of polypeptides and polynucleotides of the invention are associated with the presence of small imaginal discs (block to proliferation). Phenotypes associated with Category 5 polypeptides and polynucleotides include any one or more of the following, singly or in combination: 2nd chromosome, small imaginal discs.

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The polypeptides and polynucleotides of the invention may also be categorised according to their function, or their putative function.

For example, the polypeptides described here preferably comprise, and the polynucleotides described here are ones which preferably encode polypeptides comprising, any one or more of the following: a CBP activator protein; a CCR4-associated regulator of polymerase II transcription; a CTP synthase (CTPS); a Cyclin specific ubiquitin conjugating enzyme; a DNA packaging protein; a DNA repair protein; a DNA-binding protein involved in chromosomal organisation; a DNase IV; a EIF4G2 translation initiation factor; a eukaryotic translation initiation factor 6; a Ecdysone-induced protein 78C; a Egf2 translation factor; a G protein-coupled receptor kinase 7; a GTPase exchange factor; a phosphatidylinositol transfer protein beta isoform; a His-rich protein; a Lk6 kinase; a MAP kinase; a MAP kinase interacting kinase 1; a N-arginine dibasic convertase; a Phosphatidylinositol transfer protein; a RIP protein kinase; a RNA binding motif, single stranded interacting protein; a RNA binding protein; a RYKreceptor tyrosine kinase; a Ribosomal protein L1; a selenide, water dikinase 1; a selenium donor protein 1; a selenophosphate synthetase 1; a Sqv-7-like protein; a sugar modification protein; a protein involved in cytokinesis and signalling; a TEK tyrosine kinase; a Translation elongation

WO 01/72774

PCT/GB01/01297

factor; a UDP-galactose transporter; a v-erba related protein; a WD40 protein; a brahma protein; a calcium binding protein; a cell adhesion protein; a chaperone; a chromodomain helicase DNA binding protein; a chromodomain-helicase-DNA-binding protein; a coiled coil protein with ubiquitin like domain; a component of the 19S proteasome regulatory particle; a couch potato RNA binding protein; a cytidine 5-prime triphosphate synthetasea; 5 a cytoskeletal structural protein; a death domain containing protein; a developmentally expressed in axons of the CNS; a diacylglycerol-activated/phosholipid dependent protein kinase C inhibitor; a diazepam binding inhibitor; a diphosphate kinase; a dodecasattelite DNA binding protein; a doughnut protein tyrosine kinase; an elongation factor 2; a 10 endoplasmic reticulum ATPase; a eukaryotic translation initiation factor 4E binding protein 2; a factor involved in axon guidance; a fatty-acid-Coenzyme A ligase; a flap structure-specific endonuclease 1; a protein involved in the formation of the contractile ring and the initiation of cytokinesis; a glucose-6-phosphate transporter; a glycoprotein glucosyltransferase; a growth factor; a transmembrane receptor protein tyrosine kinase involved in cell growth and maintenance; a guanyl-nucleotide exchange factor involved in 15 signal transduction; a heat shock protein; a helicase; a high density lipoprotein binding protein; a histone acetyl transferase transcriptional activator; a histone acetyltransferase; a histone acetyltransferase GCN5; a protein involved in development of the abdomen (embryos); a protein involved in the development of the imaginal discs (larvae or pupae); a kinesin like protein 67a; a ligand-dependent nuclear receptor; a ligand-dependent nuclear 20 receptor; a lola-like specific RNA polymerase II transcription factor; a matrix associated protein; a membrane glycoprotein; a mitotic heterochromatin fragment clone CH(2)6; a motor protein; a motor protein involved in cytoskeleton organization; a mushroom body RNA binding protein; a myosin like proteins; a nemo-like kinase; a non-ATPase protein; a nuclear receptor NR1E1; a nucleic acid binding protein; a nucleoside diphosphate kinase 25 (NBR-A); a oly(rC)-binding protein 2 (hnRNP-E1); a peroxisome biogenesis factor 1; a phosopholipid transporter involved in lipid metabolism; a phosphatase or enhancer of Pi uptake protein; a protease; a proteasome regulatory particle; a protein involved in cytoskeleton organization and/or biogenesis; a protein kinase associated with 30 microtubules; a protein kinase mitogen-activated 7; a protein serine/threonine kinase involved in cell cycle, possibly targeted to cytoskeleton; a protein serine/threonine kinase involved in eye morphogenesis; a protein which associates with cdc25 phosphatase; a

protein which induces apoptosis; a ribonuclease P; a ribonuclease P protein subunit p29; a ser/thr phosphatase; a signal transduction protein; a signal transport protein; a sin3associated polypeptide; a single stranded DNA/RNA binding protein; a sodium-dependent dicarboxylate transporters; a ssDNA/RNA binding proteins; a striatin, calmodulin-binding protein (STRN); a structural protein of ribosome involved in protein biosynthesis; a subtelomeric heterochromatin repeats; a sugar acetylase; a sugar modification protein; a suppresspr of ras; a tRNA processing enzyme Ribonuclease P protein subunit; a thyroid hormone responsive gene; a tie receptor protein tyrosine kinase; a transacylase; a transcription factor; a transcription factor involved in chromatin remodelling; a transcriptional regulation of c-myc expression; a transcriptional regulator; a transcriptional regulators/telomeric silencing; a translation initiation factor; a tumor metastasis inhibitor; a tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein; a ubiquitin carrier protein; a ubiquitin-conjugating enzyme; a ugtUDP-glucose-glycoprotein glucosyltransferase; a zinc finger protein; an RNA polymerase II transcription factor; an acetylcholinesterase (YT blood group) precursor; an actin binding protein; an actin dependent regulator of chromatin; an acyl-CoA-binding protein; an alanine:glyoxylate aminotransferase; an alpha esterase; an ankyrin protein; an imitation-SWI protein; and an integrin beta 4 binding protein.

POLYPEPTIDES

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It will be understood that polypeptides of the invention are not limited to polypeptides having the amino acid sequence set out in Examples 1 to 70 or fragments thereof but also include homologous sequences obtained from any source, for example related viral/bacterial proteins, cellular homologues and synthetic peptides, as well as variants or derivatives thereof.

Thus polypeptides of the invention also include those encoding homologues from other species including animals such as mammals (e.g. mice, rats or rabbits), especially primates, more especially humans. More specifically, homologues included within the scope of the invention include human homologues.

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Thus, the present invention covers variants, homologues or derivatives of the amino acid sequence set out in Examples 1 to 70, as well as variants, homologues or derivatives of the nucleotide sequence coding for the amino acid sequences of the present invention.

In the context of the present invention, a homologous sequence is taken to include an amino acid sequence which is at least 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% identical, preferably at least 95 or 98% identical at the amino acid level over at least 50 or 100, preferably 200, 300, 400 or 500 amino acids with any one of the polypeptide sequences shown in the Examples. In particular, homology should typically be considered with respect to those regions of the sequence known to be essential for protein function rather than non-essential neighbouring sequences. This is especially important when considering homologous sequences from distantly related organisms.

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Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These publicly and commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues (for example less than 50 contiguous amino acids).

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus

potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

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However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid – Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each

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pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The terms "variant" or "derivative" in relation to the amino acid sequences of the present invention includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acids from or to the sequence providing the resultant amino acid sequence retains substantially the same activity as the unmodified sequence, preferably having at least the same activity as the polypeptides presented in the sequence listings in the Examples.

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Polypeptides having the amino acid sequence shown in the Examples, or fragments or homologues thereof may be modified for use in the present invention. Typically, modifications are made that maintain the biological activity of the sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions provided that the modified sequence retains the biological activity of the unmodified sequence. Alternatively, modifications may be made to deliberately inactivate one or more functional domains of the polypeptides of the invention. Amino acid substitutions may include the use of non-naturally occurring analogues, for example to increase blood plasma half-life of a therapeutically administered polypeptide.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

16

| ALIPHATIC | Non-polar | GAP |
|-----------|-------------------|------|
| | | ILV |
| | Polar - uncharged | CSTM |
| | | NQ |
| | Polar - charged | DE |
| | | KR |
| AROMATIC | | HFWY |

Polypeptides of the invention also include fragments of the full length sequences mentioned above. Preferably said fragments comprise at least one epitope. Methods of identifying epitopes are well known in the art. Fragments will typically comprise at least 6 amino acids, more preferably at least 10, 20, 30, 50 or 100 amino acids.

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Proteins of the invention are typically made by recombinant means, for example as described below. However they may also be made by synthetic means using techniques well known to skilled persons such as solid phase synthesis. Proteins of the invention may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and β -galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the function of the protein of interest sequence. Proteins of the invention may also be obtained by purification of cell extracts from animal cells.

Proteins of the invention may be in a substantially isolated form. It will be understood that the protein may be mixed with carriers or diluents which will not interfere with the intended purpose of the protein and still be regarded as substantially isolated. A protein of the invention may also be in a substantially purified form, in which case it will generally comprise the protein in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the protein in the preparation is a protein of the invention.

WO 01/72774

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A polypeptide of the invention may be labeled with a revealing label. The revealing label may be any suitable label which allows the polypeptide to be detected. Suitable labels include radioisotopes, e.g. ¹²⁵I, enzymes, antibodies, polynucleotides and linkers such as biotin. Labeled polypeptides of the invention may be used in diagnostic procedures such as immunoassays to determine the amount of a polypeptide of the invention in a sample. Polypeptides or labeled polypeptides of the invention may also be used in serological or cell-mediated immune assays for the detection of immune reactivity to said polypeptides in animals and humans using standard protocols.

A polypeptide or labeled polypeptide of the invention or fragment thereof may also be fixed to a solid phase, for example the surface of an immunoassay well or dipstick. Such labeled and/or immobilised polypeptides may be packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like. Such polypeptides and kits may be used in methods of detection of antibodies to the polypeptides or their allelic or species variants by immunoassay.

Immunoassay methods are well known in the art and will generally comprise: (a) providing a polypeptide comprising an epitope bindable by an antibody against said protein; (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

Polypeptides of the invention may be used in *in vitro* or *in vivo* cell culture systems to study the role of their corresponding genes and homologues thereof in cell function, including their function in disease. For example, truncated or modified polypeptides may be introduced into a cell to disrupt the normal functions which occur in the cell. The polypeptides of the invention may be introduced into the cell by *in situ* expression of the polypeptide from a recombinant expression vector (see below). The expression vector optionally carries an inducible promoter to control the expression of the polypeptide.

The use of appropriate host cells, such as insect cells or mammalian cells, is expected to provide for such post-translational modifications (e.g. myristolation,

18

glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Such cell culture systems in which polypeptides of the invention are expressed may be used in assay systems to identify candidate substances which interfere with or enhance the functions of the polypeptides of the invention in the cell.

POLYNUCLEOTIDES

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Polynucleotides of the invention include polynucleotides that comprise any one or more of the nucleic acid sequences set out in Examples 1 to 70 and fragments thereof. Polynucleotides of the invention also include polynucleotides encoding the polypeptides of the invention. It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

Polynucleotides of the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of the invention.

The terms "variant", "homologue" or "derivative" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the

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sequence. Preferably said variant, homologues or derivatives code for a polypeptide having biological activity.

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As indicated above, with respect to sequence homology, preferably there is at least 50 or 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown in the sequence listing herein. More preferably there is at least 95%, more preferably at least 98%, homology. Nucleotide homology comparisons may be conducted as described above. A preferred sequence comparison program is the GCG Wisconsin Bestfit program described above. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch. The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

The present invention also encompasses nucleotide sequences that are capable of hybridising selectively to the sequences presented herein, or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences are preferably at least 15 nucleotides in length, more preferably at least 20, 30, 40 or 50 nucleotides in length.

The term "hybridization" as used herein shall include "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" as well as the process of amplification as carried out in polymerase chain reaction technologies.

Polynucleotides of the invention capable of selectively hybridising to the nucleotide sequences presented herein, or to their complement, will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% or 98% homologous to the corresponding nucleotide sequences presented herein over a region of at least 20, preferably at least 25 or 30, for instance at least 40, 60 or 100 or more contiguous nucleotides.

The term "selectively hybridizable" means that the polynucleotide used as a probe is used under conditions where a target polynucleotide of the invention is found to hybridize to the probe at a level significantly above background. The background

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hybridization may occur because of other polynucleotides present, for example, in the cDNA or genomic DNA library being screening. In this event, background implies a level of signal generated by interaction between the probe and a non-specific DNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target DNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ³²P.

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Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na₃ Citrate pH 7.0).

Where the polynucleotide of the invention is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present invention. Where the polynucleotide is single-stranded, it is to be understood that the complementary sequence of that polynucleotide is also included within the scope of the present invention.

Polynucleotides which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways.

Other variants of the sequences described herein may be obtained for example by probing

21

DNA libraries made from a range of individuals, for example individuals from different populations. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the Examples. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of any on of the sequences shown in the Examples under conditions of medium to high stringency. The nucleotide sequences of the human homologues described in the Examples, may preferably be used to identify other primate/mammalian homologues since nucleotide homology between human sequences and mammalian sequences is likely to be higher than is the case for the *Drosophila* sequences identified herein.

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Similar considerations apply to obtaining species homologues and allelic variants of the polypeptide or nucleotide sequences of the invention.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used.

The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences. It will be appreciated by the skilled person that overall nucleotide homology between sequences from distantly related organisms is likely to be very low and thus in these situations degenerate PCR may be the method of choice rather than screening libraries with labeled fragments the sequences disclosed in the Examples.

22

In addition, homologous sequences may be identified by searching nucleotide and/or protein databases using search algorithms such as the BLAST suite of programs. This approach is described in the Examples.

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Alternatively, such polynucleotides may be obtained by site directed mutagenesis of characterised sequences, such as the sequences disclosed in the Examples. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides. For example, further changes may be desirable to represent particular coding changes found in the sequences disclosed in the Examples which give rise to mutant genes which have lost their regulatory function. Probes based on such changes can be used as diagnostic probes to detect such mutants.

Polynucleotides of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labeled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 8, 9, 10, or 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides of the invention as used herein.

Polynucleotides such as a DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

23

Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the lipid targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector

Polynucleotides or primers of the invention may carry a revealing label. Suitable labels include radioisotopes such as ³²P or ³⁵S, enzyme labels, or other protein labels such as biotin. Such labels may be added to polynucleotides or primers of the invention and may be detected using by techniques known *per se*.

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Polynucleotides or primers of the invention or fragments thereof labeled or unlabeled may be used by a person skilled in the art in nucleic acid-based tests for detecting or sequencing polynucleotides of the invention in the human or animal body.

Such tests for detecting generally comprise bringing a biological sample containing DNA or RNA into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and detecting any duplex formed between the probe and nucleic acid in the sample. Such detection may be achieved using techniques such as PCR or by immobilising the probe on a solid support, removing nucleic acid in the sample which is not hybridised to the probe, and then detecting nucleic acid which has hybridised to the probe. Alternatively, the sample nucleic acid may be immobilised on a solid support, and the amount of probe bound to such a support can be detected. Suitable assay methods of this and other formats can be found in for example WO89/03891 and WO90/13667.

24

Tests for sequencing nucleotides of the invention include bringing a biological sample containing target DNA or RNA into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and determining the sequence by, for example the Sanger dideoxy chain termination method (see Sambrook *et al.*).

Such a method generally comprises elongating, in the presence of suitable reagents, the primer by synthesis of a strand complementary to the target DNA or RNA and selectively terminating the elongation reaction at one or more of an A, C, G or T/U residue; allowing strand elongation and termination reaction to occur; separating out according to size the elongated products to determine the sequence of the nucleotides at which selective termination has occurred. Suitable reagents include a DNA polymerase enzyme, the deoxynucleotides dATP, dCTP, dGTP and dTTP, a buffer and ATP. Dideoxynucleotides are used for selective termination.

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Tests for detecting or sequencing nucleotides of the invention in a biological sample may be used to determine particular sequences within cells in individuals who have, or are suspected to have, an altered gene sequence, for example within cancer cells including leukaemia cells and solid tumours such as breast, ovary, lung, colon, pancreas, testes, liver, brain, muscle and bone tumours. Cells from patients suffering from a proliferative disease may also be tested in the same way.

In addition, the identification of the genes described in the Examples will allow the role of these genes in hereditary diseases to be investigated. In general, this will involve establishing the status of the gene (e.g. using PCR sequence analysis), in cells derived from animals or humans with, for example, neurological disorders or neoplasms.

The probes of the invention may conveniently be packaged in the form of a test kit
in a suitable container. In such kits the probe may be bound to a solid support where the
assay format for which the kit is designed requires such binding. The kit may also contain
suitable reagents for treating the sample to be probed, hybridising the probe to nucleic acid
in the sample, control reagents, instructions, and the like.

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NUCLEIC ACID VECTORS

Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells include bacteria such as *E. coli*, yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

Preferably, a polynucleotide of the invention in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

Vectors of the invention may be transformed or transfected into a suitable host cell as described below to provide for expression of a protein of the invention. This process may comprise culturing a host cell transformed with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the protein, and optionally recovering the expressed protein. Vectors will be chosen that are compatible with the host cell used.

The vectors may be for example, plasmid or virus vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and

26

optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used, for example, to transfect or transform a host cell.

Control sequences operably linked to sequences encoding the polypeptide of the invention include promoters/enhancers and other expression regulation signals. These control sequences may be selected to be compatible with the host cell for which the expression vector is designed to be used in. The term promoter is well-known in the art and encompasses nucleic acid regions ranging in size and complexity from minimal 10 promoters to promoters including upstream elements and enhancers.

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The promoter is typically selected from promoters which are functional in mammalian cells, although prokaryotic promoters and promoters functional in other eukaryotic cells, such as insect cells, may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as promoters of α -actin, β -actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase). They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters may also be

27

used comprising sequence elements from two or more different promoters described above.

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides may also be produced by synthetic means. Such antisense polynucleotides may be used in a method of controlling the levels of RNAs transcribed from genes comprising any one of the polynucleotides of the invention.

HOST CELLS

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Vectors and polynucleotides of the invention may be introduced into host cells for the purpose of replicating the vectors/polynucleotides and/or expressing the polypeptides of the invention encoded by the polynucleotides of the invention. Although the polypeptides of the invention may be produced using prokaryotic cells as host cells, it is preferred to use eukaryotic cells, for example yeast, insect or mammalian cells, in particular mammalian cells.

Vectors/polynucleotides of the invention may be introduced into suitable host cells using a variety of techniques known in the art, such as transfection, transformation and electroporation. Where vectors/polynucleotides of the invention are to be administered to animals, several techniques are known in the art, for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses, direct injection of nucleic acids and biolistic transformation.

PROTEIN EXPRESSION AND PURIFICATION

Host cells comprising polynucleotides of the invention may be used to express polypeptides of the invention. Host cells may be cultured under suitable conditions which allow expression of the proteins of the invention. Expression of the polypeptides of the invention may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, protein

production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG.

Polypeptides of the invention can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption.

Polypeptides of the invention may also be produced recombinantly in an *in vitro* cell-free system, such as the TnTTM (Promega) rabbit reticulocyte system.

ANTIBODIES

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The invention also provides monoclonal or polyclonal antibodies to polypeptides of the invention or fragments thereof. Thus, the present invention further provides a process for the production of monoclonal or polyclonal antibodies to polypeptides of the invention.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide bearing an epitope(s) from a polypeptide of the invention. Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an epitope from a polypeptide of the invention contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against epitopes in the polypeptides of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against epitopes in the

29

polypeptides of the invention can be screened for various properties; i.e., for isotype and epitope affinity.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art.

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Antibodies, both monoclonal and polyclonal, which are directed against epitopes from polypeptides of the invention are particularly useful in diagnosis, and those which are neutralising are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the agent against which protection is desired.

Techniques for raising anti-idiotype antibodies are known in the art. These antiidiotype antibodies may also be useful in therapy.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies (scFv). Furthermore, the antibodies and fragments thereof may be humanised antibodies, for example as described in EP-A-239400.

Antibodies may be used in method of detecting polypeptides of the invention present in biological samples by a method which comprises: (a) providing an antibody of the invention; (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said antibody is formed.

Suitable samples include extracts tissues such as brain, breast, ovary, lung, colon,
pancreas, testes, liver, muscle and bone tissues or from neoplastic growths derived from such tissues.

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Antibodies of the invention may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

ASSAYS

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The present invention provides assays that are suitable for identifying substances which bind to polypeptides of the invention and which affect, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, cytokinesis functions, chromatin binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

In addition, assays suitable for identifying substances that interfere with binding of polypeptides of the invention, where appropriate, to components of cell division cycle machinery. This includes not only components such as microtubules but also signalling components and regulatory components as indicated above. Such assays are typically *in vitro*. Assays are also provided that test the effects of candidate substances identified in preliminary *in vitro* assays on intact cells in whole cell assays. The assays described below, or any suitable assay as known in the art, may be used to identify these substances.

According to one aspect of the invention, therefore, we provide one or more substances identified by any of the assays described below, *viz*, mitosis assays, meiotic assays, polypeptide binding assays, microtubule binding/polymerisation assays, microtubule purification and binding assays, microtubule organising centre (MTOC) nucleation activity assays, motor protein assay, assay for spindle assembly and function,

assays for dna replication, chromosome condensation assays, kinase assays, kinase inhibitor assays, and whole cell assays, each as described in further detail below.

CANDIDATE SUBSTANCES

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A substance that inhibits cell cycle progression as a result of an interaction with a polypeptide of the invention may do so in several ways. For example, if the substance inhibits cell division, mitosis and/or meiosis, it may directly disrupt the binding of a polypeptide of the invention to a component of the spindle apparatus by, for example, binding to the polypeptide and masking or altering the site of interaction with the other component. A substance which inhibits DNA replication may do so by inhibiting the phosphorylation or de-phosphorylation of proteins involved in replication. For example, it is known that the kinase inhibitor 6-DMAP (6-dimethylaminopurine) prevents the initiation of replication (Blow, JJ, 1993, *J Cell Biol*122,993-1002). Candidate substances of this type may conveniently be preliminarily screened by *in vitro* binding assays as, for example, described below and then tested, for example in a whole cell assay as described below. Examples of candidate substances include antibodies which recognise a polypeptide of the invention.

A substance which can bind directly to a polypeptide of the invention may also inhibit its function in cell cycle progression by altering its subcellular localisation and hence its ability to interact with its normal substrate. The substance may alter the subcellular localisation of the polypeptide by directly binding to it, or by indirectly disrupting the interaction of the polypeptide with another component. For example, it is known that interaction between the p68 and p180 subunits of DNA polymerase alphaprimase enzyme is necessary in order for p180 to translocate into the nucleus (Mizuno et al (1998) *Mol Cell Biol*18,3552-62), and accordingly, a substance which disrupts the interaction between p68 and p180 will affect nuclear translocation and hence activity of the primase. A substance which affects mitosis may do so by preventing the polypeptide and components of the mitotic apparatus from coming into contact within the cell.

These substances may be tested using, for example the whole cells assays described below. Non-functional homologues of a polypeptide of the invention may also be tested

32

for inhibition of cell cycle progression since they may compete with the wild type protein for binding to components of the cell division cycle machinery whilst being incapable of the normal functions of the protein or block the function of the protein bound to the cell division cycle machinery. Such non-functional homologues may include naturally occurring mutants and modified sequences or fragments thereof.

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Alternatively, instead of preventing the association of the components directly, the substance may suppress the biologically available amount of a polypeptide of the invention. This may be by inhibiting expression of the component, for example at the level of transcription, transcript stability, translation or post-translational stability. An example of such a substance would be antisense RNA or double-stranded interfering RNA sequences which suppresses the amount of mRNA biosynthesis.

Suitable candidate substances include peptides, especially of from about 5 to 30 or 10 to 25 amino acids in size, based on the sequence of the polypeptides described in the Examples, or variants of such peptides in which one or more residues have been substituted. Peptides from panels of peptides comprising random sequences or sequences which have been varied consistently to provide a maximally diverse panel of peptides may be used.

Suitable candidate substances also include antibody products (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies) which are specific for a polypeptide of the invention. Furthermore, combinatorial libraries, peptide and peptide mimetics, defined chemical entities, oligonucleotides, and natural product libraries may be screened for activity as inhibitors of binding of a polypeptide of the invention to the cell division cycle machinery, for example mitotic/meiotic apparatus (such as microtubules). The candidate substances may be used in an initial screen in batches of, for example 10 substances per reaction, and the substances of those batches which show inhibition tested individually. Candidate substances which show activity in *in vitro* screens such as those described below can then be tested in whole cell systems, such as mammalian cells which will be exposed to the inhibitor and tested for inhibition of any of the stages of the cell cycle.

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Polypeptide Binding Assays

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One type of assay for identifying substances that bind to a polypeptide of the invention involves contacting a polypeptide of the invention, which is immobilised on a solid support, with a non-immobilised candidate substance determining whether and/or to what extent the polypeptide of the invention and candidate substance bind to each other. Alternatively, the candidate substance may be immobilised and the polypeptide of the invention non-immobilised.

In a preferred assay method, the polypeptide of the invention is immobilised on beads such as agarose beads. Typically this is achieved by expressing the component as a GST-fusion protein in bacteria, yeast or higher eukaryotic cell lines and purifying the GST-fusion protein from crude cell extracts using glutathione-agarose beads (Smith and Johnson, 1988). As a control, binding of the candidate substance, which is not a GST-fusion protein, to the immobilised polypeptide of the invention is determined in the absence of the polypeptide of the invention. The binding of the candidate substance to the immobilised polypeptide of the invention is then determined. This type of assay is known in the art as a GST pulldown assay. Again, the candidate substance may be immobilised and the polypeptide of the invention non-immobilised.

It is also possible to perform this type of assay using different affinity purification systems for immobilising one of the components, for example Ni-NTA agarose and histidine-tagged components.

Binding of the polypeptide of the invention to the candidate substance may be determined by a variety of methods well-known in the art. For example, the non-immobilised component may be labeled (with for example, a radioactive label, an epitope tag or an enzyme-antibody conjugate). Alternatively, binding may be determined by immunological detection techniques. For example, the reaction mixture can be Western blotted and the blot probed with an antibody that detects the non-immobilised component. ELISA techniques may also be used.

Candidate substances are typically added to a final concentration of from 1 to 1000 nmol/ml, more preferably from 1 to 100 nmol/ml. In the case of antibodies, the final concentration used is typically from 100 to 500 μ g/ml, more preferably from 200 to 300 μ g/ml.

Microtubule Binding/Polymerisation Assays

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In the case of polypeptides of the invention that bind to microtubules, another type of *in vitro* assay involves determining whether a candidate substance modulates binding of a polypeptide of the invention to microtubules. Such an assay typically comprises contacting a polypeptide of the invention with microtubules in the presence or absence of the candidate substance and determining if the candidate substance has an affect on the binding of the polypeptide of the invention to the microtubules. This assay can also be used in the absence of candidate substances to confirm that a polypeptide of the invention does indeed bind to microtubules. Microtubules may be prepared and assays conducted as follows:

Microtubule Purification and Binding Assays

Microtubules are purified from 0-3h-old *Drosophila* embryos essentially as described previously (Saunders, *et al.*, 1997). About 3 ml of embryos are homogenized with a Dounce homogenizer in 2 volumes of ice-cold lysis buffer (0.1 M Pipes/NaOH, pH6.6, 5 mM EGTA, 1 mM MgSO4, 0.9 M glycerol, 1 mM DTT, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin). The microtubules are depolymerized by incubation on ice for 15 min, and the extract is then centrifuged at 16,000 g for 30 min at 4°C. The supernatant is recentrifuged at 135,000 g for 90 min at 4°C. Microtubules in this later supernatant are polymerized by addition of GTP to 1 mM and taxol to 20 μM and incubation at room temperature for 30 min. A 3 ml aliquot of the extract is layered on top of 3 ml 15% sucrose cushion prepared in lysis buffer. After centrifuging at 54,000g for 30 min at 20°C using a swing out rotor, the microtubule pellet is resuspended in lysis buffer.

Microtubule overlay assays are performed as previously described (Saunders et al., 1997). 500 ng per lane of recombinant Asp, recombinant polypeptide, and bovine serum albumin (BSA, Sigma) are fractionated by 10% SDS-PAGE and blotted onto PVDF

Super Signal detection system (Pierce).

WO 01/72774

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membranes (Millipore). The membranes are preincubated in TBST (50mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% low fat powdered milk (LFPM) for 1 h and then washed 3 times for 15 min in lysis buffer. The filters are then incubated for 30 minutes in lysis buffer containing either 1 mM GDP, 1 mM GTP, or 1 mM GTP-γ-S. MAP-free bovine brain tubulin (Molecular Probes) is polymerised at a concentration of 2 μg/ml in lysis buffer by addition of GTP to a final concentration of 1 mM and incubated at 37° C for 30 min. The nucleotide solutions are removed and the buffer containing polymerised microtubules added to the membanes for incubation for 1h at 37° C with addition of taxol at a final concentration of 10 μM for the final 30 min. The blots are then washed 3 times with TBST and the bound tubulin detected using standard Western blot procedures using anti-β-tubulin antibodies (Boehringer Manheim) at $2.5 \,\mu$ g/ml and the

It may be desirable in one embodiment of this type of assay to deplete the polypeptide of the invention from cell extracts used to produce polymerise microtubules. This may, for example, be achieved by the use of suitable antibodies.

A simple extension to this type of assay would be to test the effects of purified polypeptide of the invention upon the ability of tubulin to polymerise *in vitro* (for example, as used by Andersen and Karsenti, 1997) in the presence or absence of a candidate substance (typically added at the concentrations described above). *Xenopus* cell-free extracts may conveniently be used, for example as a source of tubulin.

Microtubule Organising Centre (MTOC) Nucleation Activity Assays

Candidate substances, for example those identified using the binding assays described above, may be screening using a microtubule organising centre nucleation activity assay to determine if they are capable of disrupting MTOCs as measured by, for example, aster formation. This assay in its simplest form comprises adding the candidate substance to a cellular extract which in the absence of the candidate substance has microtubule organising centre nucleation activity resulting in formation of asters.

In a preferred embodiment, the assay system comprises (i) a polypeptide of the invention and (ii) components required for microtubule organising centre nucleation activity except for functional polypeptide of the invention, which is typically removed by immunodepletion (or by the use of extracts from mutant cells). The components themselves are typically in two parts such that microtubule nucleation does not occur until the two parts are mixed. The polypeptide of the invention may be present in one of the two parts initially or added subsequently prior to mixing of the two parts.

Subsequently, the polypeptide of the invention and candidate substance are added to the component mix and microtubule nucleation from centrosomes measured, for example by immunostaining for the polypeptide of the invention and visualising aster formation by immuno-fluorescence microscopy. The polypeptide of the invention may be preincubated with the candidate substance before addition to the component mix. Alternatively, both the polypeptide of the invention and the candidate substance may be added directly to the component mix, simultaneously or sequentially in either order.

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The components required for microtubule organising centre formation typically include salt-stripped centrosomes prepared as described in Moritz *et al.*, 1998. Stripping centrosome preparations with 2 M KI removes the centrosome proteins CP60, CP190, CNN and γ -tubulin. Of these, neither CP60 nor CP190 appear to be required for microtubule nucleation. The other minimal components are typically provided as a depleted cellular extract, or conveniently, as a cellular extract from cells with a nonfunctional variant of a polypeptide of the invention. Typically, labeled tubulin (usually β -tubulin) is also added to assist in visualising aster formation.

Alternatively, partially purified centrosomes that have not been salt-stripped may be used as part of the components. In this case, only tubulin, preferably labeled tubulin is required to complete the component mix.

Candidate substances are typically added to a final concentration of from 1 to 1000 nmol/ml, more preferably from 1 to 100 nmol/ml. In the case of antibodies, the final

37

concentration used is typically from 100 to 500 μ g/ml, more preferably from 200 to 300 μ g/ml.

The degree of inhibition of aster formation by the candidate substance may be determined by measuring the number of normal asters per unit area for control untreated cell preparation and measuring the number of normal asters per unit area for cells treated with the candidate substance and comparing the result. Typically, a candidate substance is considered to be capable of disrupting MTOC integrity if the treated cell preparations have less than 50%, preferably less than 40, 30, 20 or 10% of the number of asters found in untreated cells preparations. It may also be desirable to stain cells for γ -tubulin to determine the maximum number of possible MTOCs present to allow normalisation between samples.

Motor Protein Assay

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Polypeptides of the invention may interact with motor proteins such as the Eg5-like motor protein *in vitro*. The effects of candidate substances on such a process may be determined using assays wherein the motor protein is immobilised on coverslips. Rhodamine labeled microtubules are then added and their translocation can be followed by fluorescent microscopy. The effect of candidate substances may thus be determined by comparing the extent and/or rate of translocation in the presence and absence of the candidate substance. Generally, candidate substances known to bind to a polypeptide of the invention, would be tested in this assay. Alternatively, a high throughput assay may be used to identify modulators of motor proteins and the resulting identified substances tested for affects on a polypeptide of the invention as described above.

Typically this assay uses microtubules stabilised by taxol (e.g. Howard and Hyman 1993; Chandra and Endow, 1993 – both chapters in "Motility Assays for Motor Proteins" Ed Jon Scholey, pub Academic Press). If however, a polypeptide of the invention were to promote stable polymerisation of microtubules (see above) then these microtubules could be used directly in motility assays.

38

Simple protein-protein binding assays as described above, using a motor protein and a polypeptide of the invention may also be used to confirm that the polypeptide of the invention binds to the motor protein, typically prior to testing the effect of candidate substances on that interaction.

Assay for Spindle Assembly and Function

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A further assay to investigate the function of polypeptide of the invention and the effect of candidate substances on those functions is an assay which measures spindle assembly and function. Typically, such assays are performed using *Xenopus* cell free systems, where two types of spindle assembly are possible. In the "half spindle" assembly pathway, a cytoplasmic extract of CSF arrested oocytes is mixed with sperm chromatin. The half spindles that form subsequently fuse together. A more physiological method is to induce CSF arrested extracts to enter interphase by addition of calcium, whereupon the DNA replicates and kinetochores form. Addition of fresh CSF arrested extract then induces mitosis with centrosome duplication and spindle formation (for discussion of these systems see Tournebize and Heald, 1996).

Again, generally, candidate substances known to bind to a polypeptide of the invention, or non-functional polypeptide variants of the invention, would be tested in this assay. Alternatively, a high throughput assay may be used to identify modulators of spindle formation and function and the resulting identified substances tested for affects binding of the polypeptide of the invention as described above.

Assays for DNA Replication

Another assay to investigate the function of polypeptide of the invention and the effect of candidate substances on those functions is as assay for replication of DNA. A number of cell free systems have been developed to assay DNA replication. These can be used to assay the ability of a substance to prevent or inhibit DNA replication, by conducting the assay in the presence of the substance. Suitable cell-free assay systems include, for example the SV-40 assay (Li and Kelly, 1984, *Proc. Natl. Acad. Sci USA* 81, 6973-6977; Waga and Stillman, 1994, *Nature* 369, 207-212.). A *Drosophila* cell free replication system, for example as described by Crevel and Cotteril (1991), *EMBO J.* 10,

WO 01/72774

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4361-4369, may also be used. A preferred assay is a cell free assay derived from *Xenopus* egg low speed supernatant extracts described in Blow and Laskey (1986, *Cell* 47,577-587) and Sheehan et al. (1988, *J. Cell Biol.* 106, 1-12), which measures the incorporation of nucleotides into a substrate consisting of *Xenopus* sperm DNA or HeLa nuclei. The nucleotides may be radiolabelled and incorporation assayed by scintillation counting. Alternatively and preferably, bromo-deoxy-uridine (BrdU) is used as a nucleotide substitute and replication activity measured by density substitution. The latter assay is able to distinguish genuine replication initiation events from incorporation as a result of DNA repair. The human cell-free replication assay reported by Krude, et al (1997), *Cell* 88, 109-19 may also be used to assay the effects of substances on the polypeptides of the invention.

Other In Vitro Assays

Other assays for identifying substances that bind to a polypeptide of the invention are also provided. For example, substances which affect chromosome condensation may be assayed using the *in vitro* cell free system derived from *Xenopus* eggs, as known in the art.

Substances which affect kinase activity or proteolysis activity are of interest. It is known, for example, that temporal control of ubiquitin-proteasome mediated protein degradation is critical for normal G1 and S phase progression (reviewed in Krek 1998, Curr Opin Genet Dev 8, 36-42). A number of E3 ubiquitin protein ligases, designated SCFs (Skp1-cullin-F-box protein ligase complexes), confer substrate specificity on ubiquitination reactions, while protein kinases phosphorylate substrates destined for destruction and convert them into preferred targets for ubiquitin modification catalyzed by SCFs. Furthermore, ubiquitin-mediated proteolysis due to the anaphase-promoting complex/cyclosome (APC/C) is essential for separation of sister chromatids during mitosis, and exit from mitosis (Listovsky et al., 2000, Exp Cell Res 255, 184-191).

Substances which inhibit or affect kinase activity may be identified by means of a kinase assay as known in the art, for example, by measuring incorporation of ³²P into a suitable peptide or other substrate in the presence of the candidate substance. Similarly,

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substances which inhibit or affect proteolytic activity may be assayed by detecting increased or decreased cleavage of suitable polypeptide substrates.

Assays for these and other protein or polypeptide activities are known to those skilled in the art, and may suitably be used to identify substances which bind to a polypeptide of the invention and affect its activity.

Whole Cell Assays

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Candidate substances may also be tested on whole cells for their effect on cell cycle progression, including mitosis and/or meiosis. Preferably the candidate substances have been identified by the above-described *in vitro* methods. Alternatively, rapid throughput screens for substances capable of inhibiting cell division, typically mitosis, may be used as a preliminary screen and then used in the *in vitro* assay described above to confirm that the affect is on a particular polypeptide of the invention.

The candidate substance, i.e. the test compound, may be administered to the cell in several ways. For example, it may be added directly to the cell culture medium or injected into the cell. Alternatively, in the case of polypeptide candidate substances, the cell may be transfected with a nucleic acid construct which directs expression of the polypeptide in the cell. Preferably, the expression of the polypeptide is under the control of a regulatable promoter.

Typically, an assay to determine the effect of a candidate substance identified by the method of the invention on a particular stage of the cell division cycle comprises administering the candidate substance to a cell and determining whether the substance inhibits that stage of the cell division cycle. Techniques for measuring progress through the cell cycle in a cell population are well known in the art. The extent of progress through the cell cycle in treated cells is compared with the extent of progress through the cell cycle in an untreated control cell population to determine the degree of inhibition, if any. For example, an inhibitor of mitosis or meiosis may be assayed by measuring the proportion of cells in a population which are unable to undergo mitosis/meiosis and comparing this to the proportion of cells in an untreated population.

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The concentration of candidate substances used will typically be such that the final concentration in the cells is similar to that described above for the *in vitro* assays.

A candidate substance is typically considered to be an inhibitor of a particular stage in the cell division cycle (for example, mitosis) if the proportion of cells undergoing that particular stage (i.e., mitosis) is reduced to below 50%, preferably below 40, 30, 20 or 10% of that observed in untreated control cell populations.

THERAPEUTIC USES

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Many tumours are associated with defects in cell cycle progression, for example loss of normal cell cycle control. Tumour cells may therefore exhibit rapid and often aberrant mitosis. One therapeutic approach to treating cancer may therefore be to inhibit mitosis in rapidly dividing cells. Such an approach may also be used for therapy of any proliferative disease in general. Thus, since the polypeptides of the invention appear to be required for normal cell cycle progression, they represent targets for inhibition of their functions, particularly in tumour cells and other proliferative cells.

The term proliferative disorder is used herein in a broad sense to include any disorder that requires control of the cell cycle, for example, cardiovascular disorders such as restenosis and cardiomyopathy, auto-immune disorders such as glomerulonephritis and rheumatoid arthritis, dermatological disorders such as psoriasis, anti-inflammatory, antifungal, antiparasitic disorders such as malaria, emphysema and alopecia.

One possible approach is to express anti-sense constructs directed against polynucleotides of the invention, preferably selectively in tumour cells, to inhibit gene function and prevent the tumour cell from progressing through the cell cycle. Anti-sense constructs may also be used to inhibit gene function to prevent cell cycle progression in a proliferative cell. Another approach is to use non-functional variants of polypeptides of the invention that compete with the endogenous gene product for cellular components of cell cycle machinery, resulting in inhibition of function. Alternatively, compounds identified by the assays described above as binding to a polypeptide of the invention may

be administered to tumour or proliferative cells to prevent the function of that polypeptide. This may be performed, for example, by means of gene therapy or by direct administration of the compounds. Suitable antibodies of the invention may also be used as therapeutic agents.

Alternatively, double-stranded (ds) RNA is a powerful way of interfering with gene expression in a range of organisms that has recently been shown to be successful in mammals (Wianny and Zernicka-Goetz, 2000, Nat Cell Biol 2000, 2, 70-75). Double stranded RNA corresponding to the sequence of a polynucleotide according to the invention can be introduced into or expressed in oocytes and cells of a candidate organism to interfere with cell division cycle progression.

In addition, a number of the mutations described herein exhibit aberrant meiotic phenotypes. Aberrant meiosis is an important factor in infertility since mutations that affect only meiosis and not mitosis will lead to a viable organism but one that is unable to produce viable gametes and hence reproduce. Consequently, the elucidation of genes involved in meiosis is an important step in diagnosing and preventing/treating fertility problems. Thus the polypeptides of the invention identified in mutant *Drosophila* having meiotic defects (as is clearly indicated in the Examples) may be used in methods of identifying substances that affect meiosis. In addition, these polypeptides, and corresponding polynucleotides, may be used to study meiosis and identify possible mutations that are indicative of infertility. This will be of use in diagnosing infertility problems.

ADMINISTRATION

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Substances identified or identifiable by the assay methods of the invention may preferably be combined with various components to produce compositions of the invention. Preferably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition of the invention may be administered by

43

direct injection. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration. Typically, each protein may be administered at a dose of from 0.01 to 30 mg/kg body weight, preferably from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

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Polynucleotides/vectors encoding polypeptide components (or antisense constructs) for use in inhibiting cell cycle progression, for example, inhibiting mitosis or meiosis, may be administered directly as a naked nucleic acid construct. They may further comprise flanking sequences homologous to the host cell genome. When the polynucleotides/vectors are administered as a naked nucleic acid, the amount of nucleic acid administered may typically be in the range of from 1 µg to 10 mg, preferably from 100 µg to 1 mg. It is particularly preferred to use polynucleotides/ vectors that target specifically tumour or proliferative cells, for example by virtue of suitable regulatory constructs or by the use of targeted viral vectors.

Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectamTM and transfectamTM). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

Preferably the polynucleotide, polypeptide, compound or vector described here may be conjugated, joined, linked, fused, or otherwise associated with a membrane translocation sequence.

Preferably, the polynucleotide, polypeptide, compound or vector, etc described here may be delivered into cells by being conjugated with, joined to, linked to, fused to, or otherwise associated with a protein capable of crossing the plasma membrane and/or the nuclear membrane (i.e., a membrane translocation sequence). Preferably, the substance of interest is fused or conjugated to a domain or sequence from such a protein responsible for the translocational activity. Translocation domains and sequences for example include

44

domains and sequences from the HIV-1-trans-activating protein (Tat), *Drosophila* Antennapedia homeodomain protein and the herpes simplex-1 virus VP22 protein. In a highly preferred embodiment, the substance of interest is conjugated with penetratin protein or a fragment of this. Penetratin comprises the sequence

RQIKIWFQNRRMKWKK and is described in Derossi, et al., (1994), J. Biol. Chem. 269, 10444-50; use of penetratin-drug conjugates for intracellular delivery is described in WO/00/01417. Truncated and modified forms of penetratin may also be used, as described in WO/00/29427.

Preferably the polynucleotide, polypeptide, compound or vector according to the invention is combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration.

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The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

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EXAMPLES

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Generation and Identification of Lethal, Semi-Lethal and Sterile Third

Chromosome Mutants Having Defects in Mitosis and/Or Meiosis, and Second

Chromosome Mutants Having Defects in Imaginal Disc Development By P-Element

Insertion Mutagenesis

P-element mutagenesis

Transposable elements are widely used for mutagenesis in *Drosophila* melanogaster as they couple the advantages of providing effective genetic lesions with ease of detecting disrupted genes for the purpose of molecular cloning. To achieve near saturation of the genome with mutations resulting from mobilisation of the P-lacW transposon (a P-element marked with a mini-white gene, bearing the *E. coli lacZ* gene as an enhancer trap, and an *E. coli* replicon and ampicillin resistance gene to facilitate 'plasmid rescue' of sequences at the site of the P-insertion), *Drosophila* females that are homozygous for P-lacW (inserted on the X chromosome) are crossed with males carrying the transposase source $P(\Delta 2-3)$ (Deak et al., 1997). Random transpositions of the mutator element are then 'captured' in lines lacking transposase activity. Stable, or balanced, stocks bearing single lethal P-lacW insertions are made.

More than 41,000 lines are derived, of which approximately one-half are on the third chromosome. Originally some 3100 lethal or strong semi-lethal lines (in homozygous conditions) are identified. During preliminary characterisation unstable lines and clusters of the same mutation event are eliminated leaving 2460 lines to be characterised.

Screening for Mitotic and Meiotic Defects

About half of the mutants in the collection are embryonic lethals. We have carried out cytological screens of the 1155 lines that comprise late larval lethals, pupal lethals, pharate and adult semi-lethals for defective mitosis in the developing larval CNS. This has identified 69 mutations falling into 43 complementation groups that affect all stages of the mitotic cycle. The cytological screens involve examining orcein-stained squashed preparations of the larval CNS to detect abnormal mitotic cells. In lines where defects are

identified, the larval CNS is subjected to immunostaining to identify centromeres, spindle microtubules and DNA for further examination. This leads to clarification of the mitotic defect.

As a set of common functions are essential to both mitosis and meiosis, we then identify mutations resulting in sterility and failed progression through male meiosis. This involves examining squashed preparations larval, pupal or adult testes by phase contrast microscopy. We examine "onion stage" spermatids in the 519 pupal and pharate lethal lines and 463 adult "semi-lethal" and viable lines for variations in size and number of nuclei which provides an indication of whether there have been defects in either chromosome segregation or cytokinesis, respectively. A total of 54 lines of the 519 pupal and pharate lethal lines and 22 of the adult lines show such defects. However, another 67 lines show male sterility without having onion-stage defects. 12 lines showing onion stage defects have been scored as having mitotic defects in the independent cytological screen of squashed preparations of the larval CNS. Twelve further lines with onion stage defects show female sterility and of these, 10 show maternal effect mitotic defects in syncytial embryos. Thus greater than one half of the meiotic mutants scored appear to represent cell division functions specific to male meiosis or have targeted male germ-line specific enhancer elements, thus revealing their meiotic function but in this test not their mitotic function.

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Further characterisation of testis preparations of each line by phase-contrast microscopy with and without staining with Hoechst to reveal DNA defined 6 broad categories of meiotic mutants:

8 mutants from the collection show defects in meiotic entry or at early stages in the first meiotic division (MF1-8).

18 mutants (15 complementation groups) show abnormal meiotic spindles (AB1-16). Mutants in this group almost invariably show an associated weak defect in cytokinesis, and 7 show a strong defect in spermatid differentiation. 3 of these mutants

also show mitotic defects in larval brains or in embryos derived from homozygous mutant mothers.

18 mutants (16 complementation groups) also show abnormal meiotic spindles that are strongly multipolar (MUL1-15). Three of these also show maternal effect mitotic abnormalities of multipolar spindles in syncytial embryos.

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4 mutants (3 complementation groups) show strong defects at all stages of spermatogenesis from the pre-meiotic stages to spermatid elongation stages (PL1-3). In this respect they resemble the *polo*¹ mutation.

4 mutants show segregation defects as indicated by spermatid nuclei of
heterogeneous sizes (SEG1-4). The spindles appear normal but all have what are either chromosome bridges or lagging chromosomes. One of these also shows a maternal effect.

9 mutants (7 complementation groups) show predominant cytokinesis defects. Two complementation groups also have cytokinesis defects in mitotic cells in the larval brain.

In the Examples below, the designations MF, AB, MUL, PL, SEG or CK are included in the category description where available. Further phenotype information for each mutant described in the results section is provided in the "Phenotype" field. There is considerable overlap between these categories, and it will be of much interest to distinguish between mutants in which the primary defect results in secondary consequences, and mutants that affect more than one aspect of spermatogenesis, as for example appears to be the case with *polo* mutants (Sunkel and Glover, 1988; Carmena et al, 1998).

In the Examples, lines exhibiting mitotic and meiotic phenotypes are categorised generally into four categories:

Category 1: Failure to complete cytokinesis

48

Category 2: Failure to enter M-phase

Category 3: Metaphase arrest

Category 4: Anaphase defect

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Category 5: Small Imaginal Discs (Block to Proliferation; see below)

Category 1 phenotypes are exhibited by mutations in Examples 1 to 14; while Category 2 phenotypes are exhibited by mutations in Examples 15 to 19. Category 3 phenotypes are exhibited by mutations in Examples 20 to 30, Category 4 phenotypes are exhibited by mutations in Examples 31 to 53. Mutations in Examples 54 to 74 exhibit a Category 5 phenotype.

Generation and identification of second chromosome mutants having small or no imaginal discs.

In the case of the second chromosome the flies used were from a second chromosome P-element collection established in Szeged, Hungary (Torok et al., 1993). The process of P-element insertion mutagenesis is essentially as described above. 15475 insertions were recovered, of which 2711 were lethal or semi-lethal. After elimination of clusters of identical mutants, 2399 lines representing 1748 independent lethal insertions were recovered. Lines were chosen from the second chromosome collection on the basis of having small or no imaginal discs, to indicate a disruption in cell cycle progression that leads to underdevelopment of the discs. All the second chromosome mutants referred to in the results section are noted under the "Phenotype" field as "second chromosome, small imaginal discs" and comprise Category 5.

Cytological Mapping of the P-Element Insertion Sites

The site of insertion of the P-element in each mutant line was determined by *in situ* hybridisation of P-element DNA to salivary gland polytene chromosomes as described in Saunders et al., 1989. Wandering third stage larvae were dissected and fixed as described and incubated with biotin-labeled DNA made from the *P-lacW* plasmid. After signal

detection chromosomes were stained with Giemsa and examined by microscopy and signals indicating the presence of P elements were assigned to polytene chromosome bands referring to the Bridges map (Lefevre, 1976). In the majority of cases a single P element was detected, only 10% of lines having multiple (two or three) insertions. The site of insertion is given as the "Map Position" field in the results section (for example 77B)

Plasmid Rescue of P-Elements from Mutant Drosophila Lines

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Genomic DNA was isolated from adult flies by the method of Jowett et al., 1986, and plasmid rescue from the genomic DNA was performed according to Pirrotta et al., 1986. This allows the recovery of genomic DNA adjacent to the P-element which facilitates the identification of the site of P-element insertion and of genes which may be disrupted by the insertion. Essentially, genomic DNA derived from about 200 flies was digested with a restriction enzyme known to have a site within the P-element (EcoR1 or SacII for cloning sequences to the left of the element, or XbaI, BglII, PstI or BamHI for sequences to the right of the element). The digested DNA was ligated overnight, and plasmids recovered by electroporation of the ligated DNA into *E.coli* XL1-blue competent cells. Appropriate primers from within the P-lacW sequence were used to determine the sequence of the genomic DNA flanking the element (on average, 400 bp of sequence were obtained). The rescue sequences are provided in the results section under the heading "Rescue sequence". Where more than one sequence was recovered, the orientation of each sequence is also given.

Sequence Analysis of P Element Insertion Lines

Sequences flanking the insertion site of the P-element were derived by P element rescue as described above. In some cases sequence was obtained from only one side of the insertion, while in other cases sequences were obtained from both sides of the insertion.

As a first step, each P element rescue sequence was used to search a total database of *Drosophila melanogaster* sequences (database of the Berkley *Drosophila* Genome project) using the BLASTN program (which compares a nucleic acid sequence with a nucleic acid database, (Altschul and Lipman 1990)) with default parameters.

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The search may identify a number of different types of match including Drosophila ESTs, known Drosophila genes and cloned genomic regions.

The ability to identify genes already known to be essential for cell cycle progression using this approach was confirmed, in this example, by the rescue sequence obtained from line 1324/8 which mapped to the 77B locus which was used to search the database. A BLASTN search identified a number of matching *Drosophila* ESTs, a match with the known cell cycle regulatory gene *polo* and a cloned genomic region designated CSC: AC018188. These matches are recorded in the results sections under the field headings "*Drosophila* ESTs", "*Drosophila* gene hit" and "Genomic hit, Accession No.", respectively. Any entries under "*Drosophila* gene hit" are further annotated with "(BLASTN with Rescue sequence)" to show that the match was obtained using the rescue sequence rather than a *Drosophila* EST or genomic clone ORF (see below). Accession numbers of ESTs, genes and genomic clones are provided where known. Genomic clones designations often include the Genbank designation as part of a longer designation. However the Genbank designation is always the code beginning with "AC" and followed by six digits.

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Where an EST was identified, this was subsequently used to search using the BLASTX program (default parameters) against databases of sequences from *Drosophila* and Homo sapiens (databases of the National centre for Biotechnology Information (NCBI), National Library of Medicine, National Institue of Health, USA). In the case of line 1104/16, the search identified a known human gene, phosphatidylinositol transfer protein (accession no. P48739) implying a novel function for this protein in cytokinesis. Human Homologues identified as a result of a BLASTX search using a *Drosophila* EST are shown in the results section under the heading "Human homologues" and annotated with "(BLASTX with EST)". *Drosophila* genes identified as a result of a BLASTX search using a *Drosophila* EST are shown in the results section under the heading "*Drosophila* gene hit" and annotated with "(BLASTX with EST)".

Where no *Drosophila* gene was identified using the initial BLASTN search but a matching genomic clone was found (a Bac or P1 clone often in excess of 100 kilobases), a

51

20 kilobase segment of this genomic sequence (10 kilobases either side flanking the site of the P-element insertion) was subjected to a number of analyses.

If the rescue sequence matched sequences that lie within a known gene present within the genomic clone then these are presented under the heading "Drosophila gene hit (BLASTN with Rescue sequence". The known gene sequence was then used in a BLASTX search of a human database (NCBI – see above) to identify any human homologues. These are shown in the "Human homologue" field and annotated with "(BLASTX with Drosophila gene)".

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within the genomic clone then the occurrence of ORFs within the 20 kilobase genomic segment was predicted using the Genscan programme (Burge and Karlin, 1997). Where the P-element was observed to be inserted into the coding region or within the 5' untranslated region (which we defined as within 2 kilobases of the predicted start of the coding region) we assume the P element to be capable of disrupting the expression of the predicted gene. Each predicted open reading frame (or predicted coding sequence) was then used to search *Drosophila* and human databases using the TBLASTN program (compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames) and/or the TBLASTX program (compares a nucleotide query sequence dynamically translated in all reading frames against a nucleotide sequence database dynamically translated in all reading frames) to determine whether the predicted open reading frame corresponded to a known gene. Typically, TBLASTX is only used when no matches are found using TBLASTN.

Where the TBLASTN search found a known *Drosophila* gene, then this is indicated in the results in the "*Drosophila* gene hit" field, annotated with "(TBLASTN with predicted ORF)". The *Drosophila* gene sequence was then typically used to search a human database (NCBI – see above) to identify any human homologues using BLASTX. These are shown in the "Human homologue" field and annotated with "(BLASTX with *Drosophila* gene)".

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Where the TBLASTN and/or TBLASTX search found a known human gene, then this is indicated in the results in the "Human homologue" field, annotated with "(TBLASTN (or TBLASTX) with predicted ORF)".

If the TBLASTN and/or TBLASTX search found no *Drosophila* or human genes, then it was assumed that the original ORF corresponds to a novel gene. If the TBLASTN search found no *Drosophila* genes but identified a human homologue, then it was assumed that the original ORF corresponds to a novel *Drosophila* homologue of a known human gene.

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Additional Sequence Analysis using the Annotated D. melanogaster Sequence (GadFly).

Rescue sequences were also used to search the fully annotated version of the Drosophila genome (GadFly; Adams, et al., 2000; Science 287, 2185-2195), using GlyBLAST at the Berkeley Drosophila Genome Projects web site to identify the genome segment (usually approximately 200-250 kb) containing the P-element insertion site. The graphic representation of the genomic fragment available at GadFly allows the identification of all real and theoretical genes which flank the site of insertion. Candidate genes where the P-element is either inserted within the gene or close to the 5' end of the gene were identified. In GadFly, the Drosophila genes are given the designation CG (Complete gene) and usually details of human homologues are also given. In most cases, this data confirms the data derived from the sequence analysis procedure described above, and in some cases new data is obtained. Where available both sets of data are included in the individual Examples described below. To identify further candidate human homologues, BLASTP (amino acid query sequence against amino acid database) searches with Drosophila sequences are used against the human genome project database and also the Ensembl dataset. The Ensembl dataset comprises GeneWise gene predictions using a protein template where possible or Genscan followed by BLAST confirmation via protein, cDNA or EST hits. These are matched using WUBLASTP with default parameters (Altschul et al., 1990, J Mol Biol 215, 403-10). The results are filtered to contain only potential homologues. Only matches with the identity of more than 50% and length of more than 50 amino acids are included.

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Confirmation of Cell Cycle Involvement of Candidate Genes Using Double Stranded RNA Interference (RNAi)

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P-elements usually insert into the region 5' to a *Drosophila* gene. This means that there is sometimes more than one candidate gene affected, as the P-element can insert into the 5' regions of two diverging genes (one on each DNA strand). In order to confirm which of the candidate genes is responsible for the cell cycle phenotype observed in the fly line, we use the technique of double stranded RN interference to specifically knock out gene expression in *Drosophila* cells in tissue culture (Clemens, et al., 2000, *Proc. Natl.* Acad. Sci. USA, 6499-6503). The overall strategy is to prepare double stranded RNA (dsRNA) specific to each gene of interest and to transfect this into Schneider's Drosophila line 2 to inhibit the expression of the particular gene. The dsRNA is prepared from a double stranded, gene specific PCR product with a T7 RNA polymerase binding site at each end. The PCR primers consist of 25-30 bases of gene specific sequence fused to a T7 polymerase binding site (TAATACGACTCACTATAGGGACA), and are designed to amplify a DNA fragment of around 500bp. Although this is the optimal size, the sequences in fact range from 450 bp to 650 bp. Where possible, PCR amplification is performed using genomic DNA purified from Schneider's Drosophila line 2 as a template. This is only feasible where the gene has an exon of 450 bp or more. In instances where the gene possesses only short exons of less than 450 bp, primers are designed in different exons and PCR amplification is performed using cDNA derived from Schneider's Drosophila line 2 as a template.

A sample of PCR product is analysed by horizontal gel electrophoresis and the DNA purified using a Qiagen QiaQuick PCR purification kit. 1µg of DNA is used as the template in the preparation of gene specific single stranded RNA using the Ambion T7 Megascript kit. Single stranded RNA is produced from both strands of the template and is purified and immediately annealed by heating to 90 degrees C for 15 mins followed by gradual cooling to room temperature overnight. A sample of the dsRNA is analysed by horizontal gel electrophoresis.

3μg of dsRNA is transfected into Schneider's *Drosophila* line 2 using the
 transfection agent, Transfect (Gibco) and the cells incubated for 72 hours prior to fixation.

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The DNA content of the cells is analysed by staining with propidium iodide and standard FACS analysis for DNA content. The cells in G1 and G2/S phases of the cell cycle are visualised as two separate population peaks in normal cycling S2 cells. In each experiment, Red Fluorescent Protein dsRNA is used as a negative control. In some cases the phenotype is confirmed by fixing cells on poly-lysine covered slides which are then stained for DNA using DAPI and for tubulin using an anti-tubulin antibody YL1/2 and appropriate fluorescent secondary antibody to visualise aberrant mitoses.

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PCT/GB01/01297

It should be noted that RNAi could not confirm phenotype in all cases. This is to be expected as the method relies on the ability of dsRNA to prevent new protein expression. Consequently, it is necessary that S2 cells express the specific cDNA of the gene in question, and also that the protein is turned over rapidly. It would therefore probably be difficult to sufficiently reduce levels of very stable proteins using this approach.

The layout of a typical entry in the results section is shown below. Not all fields

present in the actual results section contain information for each individual *Drosophila*line described.

TYPICAL RESULTS LAYOUT

Line ID - Drosophila line designation
Category - Description of phenotype

Reversion - R = revertant, NR = non revertant, ? = not determined

Map Position - according to the Bridges map (Lefevre, 1976).

Rescue ID

25 Rescue Sequence

[nucleotide sequence]

Genomic hit, Accession No.

30 Associated ORF

GENSCAN_predicted_peptide [results of Genscan - amino acid sequence]
GENSCAN_predicted_CDS [results of Genscan nucleotide sequence]

Drosophila Gene Hit

35 (BLASTN with rescue sequence)

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(TBLASTN (or TBLASTX) with predicted ORF) (BLASTX with EST)

Human Homologue

(BLASTX with *Drosophila* gene)
(TBLASTN (or TBLASTX) with predicted ORF)
(BLASTX with EST) *Drosophila* EST

10 Annotated *Drosophila* genome genomic segment Annotated *Drosophila* genome Complete gene candidate Human homologue of Complete gene candidate

Putative function Derived from homologies or Drosophila experimental data

Confirmation by RNAi Description of Facs analysis DNA content profile

A specific example is as follows:

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Line ID 1324/8

Category Mitotic defects in brain: metaphase arrest

(overcondensation, some circular chromosomes, no anaphases, very high mitotic index, metaphase (or less aligned) with bipolar

spindle, no CP190 staining)

Reversion R Map Position 77B

Rescue ID B1E

30 Rescue Sequence

GTTTTGCCCATCGATTGCACGAAAACCAAGCACAAAGCGGAGAACGCGCCGA AACCGTTCGATTTTTTAAATGCCAAAATGAATTGGACGTGAAGCGTCAGCTGA ATTGGTGTGCCCGTTTCGGTGGCTATCGCACACTTTCTGGTATTTATCGCGGTA TTTTGTTGAGTGTTGAACAACAAATTCTATGGCCGTTACCCTTTTGAATTTACT

- TACTGGCGTTTACTCTGTTCGAATTGAGCGCAATATTTTTTCCTATTGCTCTGC GCAACACTGTGTTTTAACCGCTATTTATTTGAAAATCTACAAAAACTAACCGTT TACATTTTTGAAATTTCCAAAAGGGTTTTCCATAAATTGAGTTTTACTAAAACC AGTCCAACGGTCCAACTTTATATTGTTAGAAGCCCCTTTTCCTAATTTGAATTG GCTTGCAAACGTTTTCCTGAATTTAAAAATACTGCCACCCTTGTTAATTGCAGG
- 40 TTTTCCGAATCCCTGATTTGTTGTTTTAAAAAGAAAATTTATTAGAAACAGCTA TCTCAACC

Genomic hit, Accession No. CSC:AC018188

Drosophila Gene Hit Polo (X63361)

45 Human Homologue BLASTX PLK-1 (P53350)

Drosophila EST several including LD11851 (AA392613) which match polo

Annotated Drosophila genome genomic segment

AE003514

56

Annotated Drosophila genome Complete gene candidate CG12306

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Human homolog of Complete gene candidate 1e-169 1709658 P53350

PLK1_HUMAN SERINE/THREONINE-PROTEIN KINASE PLK

(PLK-1)

Putative function Serine/threonine kinase known to be required for mitosis

10 Confirmation by RNAi Reduced G1 and G2/M peaks indicating fewer cycling cells,

microscopy analysis of DNA and tubulin staining identified monopolar spindles characteristic of polo mutation in

Drosophila.

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CATEGORY 1: FAILURE TO COMPLETE CYTOKINESIS

Example 1 (Category 1)

Line ID 1031/14

5 Category Mitotic defects in brain: cytokinesis defect

(polyploidy)

Reversion R Map Position 74B

10 Rescue ID 2A3B

Rescue Sequence 1

- 15 GACTAATGTGTTTAAATGTAACTTACACTAGTAACAGATCCCCATTAATAAAA GCCAAACTCTAAAAATTCTGCCACAAGTACTATTTCTCACGTAACACCTTACTA ACGGATTTCACATGATATCTACGACAAGAAACTGTTTGCTGATATAAAATTGC TATCACCGCTTTCCGTAAACACTTTTACACTGATGGATTACAAGTTCAATTAAT ACATCAACTTACCTTAACAATTTAAGACAACTAACACTCCCACAATTTAATT
- 20 CAACCTACACCGCTTGATAATCAGCTGTTCTGTACAAAAAACAATAACACTGT TAACAACAGCGCACAGTGGATAATACAGTCCTAAAGGCAATATACCCATTTG GCATTTTT

Rescue ID 2A3S

25 Rescue Sequence 2

TTCCGGGGAGAATGCTGCGATTTCGCGTCGGTAAAAATAGCAAATACTCGTTAATGTGCTGTGGGAACGCTTCCTCCCCGGCCCCAAAGTGGCCCCGAAGAAAGTGAGCAAATGTGCGCGCCGCAAGATAGTCGCCGCGAACAAACGATAGTGACGAAAGTGATTTAATTCAACTACCAGCACTCCCGCAAATACGATGAGTATGTCGCGCGG

- 30 CGGCAACACACTCTGGACTTGCAGCCGCTCCTGGCGGAGAGCGATGTCGGAA ACAGGGAGCTGGAGGAGAAGATGGGCGGATCGGCGATCGCTCTC GATGGATCCGGTTCGAAGGAGCTGAGTCACCGGGAACGCGAGGACTCGGCGTT GTTCGTCAAGAAGATCGGGAGCGCCTTGTTCTATGGCTTGTCCTCCTTCATGATT ACGGTGGTAAACAAGACGGTGCTTACCTCCTACCACTTCCCCTCGTTCCTGTTCC
- 35 TCAGCCTCGGGCAACTTACTGCTAGCATTGTGGTCCTGGGCATGGGCAAAGCGC CTGAAAATGGTGAACTTTTCCCCTTTTGCAGAGGAATACCTTCGCCAAGATCTTT CCGCTGCCACTGATATTTCTGGGAAACATGATGTTTGGACTGGGTGGCACAAAA ACCTTGAGTCTGCCCATGTTCGCAGCCCTACGAC
- 40 Genomic hit, Accession No. AC019515

Associated ORF

Genscan ORF1 predicted sequences:>15:31:57|GENSCAN predicted peptide 4|373_aa

MSMSRGGNTTLDLQPLLAESDVGNRELEEKMGGSADRSSLLDGSGSKELSHRER EDSALFVKKIGSALFYGLSSFMITVVNKTVLTSYHFPSFLFLSLGQLTASIVVLGMG KRLKLVNFPPLQRNTFAKIFPLPLIFLGNMMFGLGGTKTLSLPMFAALRRFSILMT MLLELKILGLRPSNAVQVSVYAMIGGALLAASDDLSFNMRGYIYVMITNALTASN GVYVKKKLDTSEIGKYGLMYYNSLFMFLPALALNYVTGNLDQALNFEQWNDSV FVVQFLLSCVMGFILSYSTILCTQFNSALTTTIVGCLKNICVTYLGMFIGGDYVFSW LNCIGINISVLASLLYTYVTFRRKRAPDKQDHLPSTRGENV

>15:31:57|GENSCAN predicted CDS 4|1122 bp

atgagtatgtcgcgcggcgaacacacactctggacttgcagccgctcctggcggagagcgatgtcggaaacagggagctgga 10 ggagaagatgggcggatcggcggatcggtcatcgctgctcgatggatccggttcgaaggagctgagtcaccgggaacgcgag gactcggcgttgttcgtcaagaagatcgggagcgccttgttctatggcttgtcctccttcatgattacggtggtaaacaagacggtgc tt acct cct accact tt ccct cgt tcct gtt cct cag cct cgg g caact tact gct ag catt gt gg tcct ggg cat ggg caa gc gcct tact gct accact the catter than the contract of the contract graph of the contragaaattggtgaactttccccctctgcagaggaataccttcgccagatctttccgctgccactgatatttctgggaaacatgatgtttg gactgggtggcacaaaaaccttgagtctgcccatgttcgcagccctacgacgcttctctatcctgatgaccatgctgctggagctca 15 agatccigggactgcgaccttcgaatgcggttcaggtcagcgtatacgcaatgatcggtggagcgctgctggccgcctctgatga tet g te c t tea a cat g agggget a cat et at g t g at g at each accept the accept tet g accept the tet g accept the accept the accept the accept the tet g accept the accept t20 atatctgggcatgttcattggaggcgactacgtcttctcgtggctcaactgtattgggatcaacatcagcgtgctggctagtctgctct acacgtacgtcacttttcggcggaagcgggctcccgataagcaggaccacttgcccagcacccgcggcgagaatgtctag

Human Homologue (TBLASTN with ORF1): KIAA0260 gene (D87449) and putative Sqv-7-like protein (AJ005866)

Drosophila EST CK00510 (AA140776)

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Annotated *Drosophila* genome genomic segment AE003524

Annotated *Drosophila* genome Complete gene candidate CG3874 – novel glucose-6-phosphate transporter

Human homologue of Complete gene candidate EMBL:D87449 protein

KIAA0260_id:BAA13390
gi:166578 Similar to a
C.elegans protein encoded in
cosmid C52E12 (U50135) and
Ensembl predicted gene
ENSG00000024527

Clone:AL133320 Contig:AL133320.00001

8.10E-95

Putative function Sugar modification protein similar to proteins involved in Drosophila cytokinesis and signalling

Confirmation by RNAi Marked increased G1 and S peak indicating mainly arrest in

G1

59

Example 2 (Category 1)

Line ID 1066/5

Category Male semi-sterile, Meiotic defects in testis: cytokinesis defects,

segregation defects.

(Seg-01/62)

Reversion ? Map Position 89B

10 Rescue ID F9E

Rescue Sequence

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GAATGGGGATATATTGTGGCATTGGCGAAAGGTCGCTATGC

Genomic hit, Accession No. CSC:AC019750

25 Associated ORF

>16:04:57|GENSCAN_predicted_peptide_4|418_aa
MKPIPNESKGTLAAVGDATVVHDVCTLFAVELDPYLRSSMGMRTRRAQSGALLL
QLLAVADGGFAAHICACKCRLRLPHVTCCCNRNPFKATAKAKGQAVSSTKPNQL
CFHGCCGWIITTKGETFTENSPSIMSGFAWERHSLGECVVVAGTEQILLIGRTLIGR
MSHTOTDSTSPEVVDCHSOLCGSKCKGICVSVGECVPPSCOPEDMKIVWANI AM

30 MSHTQTDSTSPFVVDCHSQLCGSKCKCICVSVGFCVRPSCQRFDMKIVWANLAM QKRFLLGAAIADMCCRNSVIWCKLQLDPVKPIDERADGSGLALVTKVCDNNNIV HYVVVAGVTGSQSRSRLQPLRSGQNESTEQWPRTKGGEGGFNNNSRNNKHSAPT QEQQELWQKQLLQDQRDDCHASGSFQSASFAETRSFTFDDTTAHSEFCFRTRAEK RRILVLLETSIKLKPDKYATSGHTRRCAIGLLHSII

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>16:04:57|GENSCAN_predicted_CDS_4|1257_bp

PCT/GB01/01297 WO 01/72774

60

gcaggaacaacaacaattctgctcccacgcaagagcagcaggaactgtggcaaaaacagctgctgcaggatcaacgagacgat tgtcatgccagtggaagcttccagtctgcgtcattcgcggagacgcgtagtttcacgttcgacgacacaaccgctcacagcgaattt tgttttcggactagagctgagaaacggcgaattttggtgcttctggaaacatcgattaaactaaaacccgataagtatgcgacaagc ggtcacactcggcgatgtgcgataggattgctgcattcgattatatag

Drosophila Gene Hit rescue sequence: mitotic heterochromatin fragment clone CH(2)6 (L36595) and subtelomeric heterochromatin repeats (L03284). TBLASTN with ORF1: nebula (nla) (AF147700) BLASTX with nebula: Down Syndrome candidate region 1-like 10 **Human Homologue** protein 2 (AF176117) Drosophila EST rescue sequence: CK01138 (AA141069) AE003712 Annotated Drosophila genome genomic segment 15 Annotated Drosophila genome Complete gene candidate CG6072 - nebula CG6046 - sap18 Human homologue of Complete gene candidate CG6072- 8e-36 'ZAKI4 a thyroid hormone responsive gene in human 20 skin fibroblasts' also known as DOWN SYNDROME CANDIDATE REGION 1-LIKE 1; DSCR1L1 EMBL:D83407 protein id:BAA11911 gi:143504 25 CG6046- 3e-45 2108210 (U96915) sin3 associated polypeptide p18 [Homo sapiens] and gi5032067 C7E479FFE9CA5774 30 |ref|NP 005861.1| sin3-associated polypeptide, 18kD [Homo sapiens] (1.90E-43)Nebula unknown function, Sap18 transcription factor 35 **Putative function**

Both show reduction in G1 and G2/S peaks indicating fewer Confirmation by RNAi cycling cells

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Line ID 234/50

Category Meiotic defects in testis: cytokinesis defects, abnormal spindles.

(Ab-02/12)

Reversion R
5 Map Position 89B

Rescue ID 2C5E

Rescue Sequence

Drosophila EST rescue sequence: CK01138 (AA141069)

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All other entries as for 1066/5.

62

Example 3 (Category 1)

Line ID

1104/16

Category

Mitotic defects in brain: cytokinesis defect

(no overcondensation of diploids, high polyploidy)

Reversion

R

Map Position

92A

Rescue ID

B5P

10 Rescue Sequence 1

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Rescue ID B5E

Rescue Sequence 2

Genomic hit, Accession No. AC006589

35 Associated ORF

Genscan: ORF1 predicted sequences

>/tmp/aaaaainga|GENSCAN_predicted_peptide_2|850_aa

MATRGANVIWFRHGLRLHDNPALLAALADKDQGIALIPVFIFDGESAGTKNVGY NRMRFLLDSLQDIDDQLQAATDGRGRLLVFEGEPAYIFRRLHEQVRLHRICIEQDC EPIWNERDESIRSLCRELNIDFVEKVSHTLWDPQLVIETNGGIPPLTYQMFLIRCTH HNGDVNGDEDTGEGEGTGGRIDWAKEGACWRAGNSDEQECQACQSVSSVIMM VLQYSNNPAHHCQLLECLMTLKHNVVKDILCVVAYGTAVSRTSAAKLLFYYWP AFNANLFDRKVLLSKLTNDLVPFTCQREHCPNSGNAEAAKVCYDHSISIAYAPDC PPPLYLCIECANEIHREHGSLEFGDILHPMQQVSMVCENKNCRSNEKSAFSICFSTE

45 CASFNGNHPIRYCSQCHSNRHNSRRGGDHVVHRSLQPAWQMDPEMQMHMVESV VSLLREAKPLNFEPGKESSSSESKKNGSGITADNISLEERQRLGRYGIWLLVGRCTP TADTPVEVLGRILSMLFHWFHVTAYSYDGFISCLVPHPPEYARVGGHWETLASRT SHLKEGLQRLICLVPYEVITSEIWDYVMPHWMEAITNDVAEKELNELKIVLSKILD PEMSPLGFDAKTMYNFVAIRFEKTTAKVQQQALHWLQILTKLEILIPLVQLFAMF GDGVRIMKYGIQHELMREKDAQSQSLAKAPKTPCKESKETKADMANPPRPPVVE DDSGNTSAISDDEAPTNRHTEFSTDAEHNLTCCILMLDILLKQMELQDVEQHMGI HTSVCENVSRLIKCMVTAARVGLSSHVCALKVPIEDIIEEEKSSRKSPPESDKEKTR DRDVSLSMAPLPIPLGPLGGFADP

>/tmp/aaaaainga|GENSCAN_predicted_CDS_2|2553_bp atggccacgcgaggggggaatgtgatttggtttcgccatggattgcgcctccatgata at10 a aga at gtgggtta caa teggat gegttteet cet ggactegt t geagga categat gat cage tacage gegeaact gat ggacgtggacgcctcctggtcttcgagggcgaaccggcttatatcttccgccggctacatgagcaagtgcgtctgcacaggatttgcataggtatcacacacgctttgggatccgcaattggtgattgagaccaatggtggcattccaccgctgacctaccaaatgttcctgatacgct15 g cac g cac caca at g g a g at g t g at g g g at a c g g g a g g a a g g a a c g g c g g a a g g at c g a c g g a g g at g g a c g g a g g a a g g a a c g g a g g a a gaggaaggggcctgttggagggcgggaaactccgacgaacaggaatgtcaggcctgccaatcagtgtcctcggtcatcatgatgctctgcgttgtggcatacggaaccgctgtttcccgcacctcggctgccaagctgctcttctactactggccagcctttaacgccaatctgttcgatcgcaaagtcctactctccaaactaaccaatgacctagtgcccttcacctgccaacgggagcactgtccgaactccggg20 aatgcggaggcagcaaaggtgtgctacgaccacagcattagcatcgcatacgcgcccgattgtccaccgcccctttacctgtgca tcgagtgcgccaacgagattcatcgggagcacggaagcctggagttcggcgacattctgcatcccatgcagcaggtatcgatggtgtgcgaaaacaagaactgtcgctccaacgagaagtccgccttctccatctgcttctccacggagtgtgccagcttcaatggcaactgtcgcaactgtcgaaactgtcgcaactgtcgaaactgtcgcaactgtcgaaactgtcgaaactgtcgcaaactgtcgaaactgaaactgtcgaaactgtcgaaactgtcgaaactgtcgaaactgtcgaaactgaaactgtcgaaactgaaactgtcgaaactcatecgatecgctactgcagecagtgccacagtaataggcacaattcccggcgaggtggcgatcacgtggtccatcgcagtctgcagcccgcctggcagatggatccagagatgcagatgcacatggtggagtcggtggtaagccttctgcgagaggcgaagccacta25 aactttgagcccggcaaggagtcctcgtcgtccgagtccaaaaagaacggctccggcatcacagctgacaatatttctctggagg a acgccagagactgggacgctatggtatctggctactggtgggtcgctgtacacccactgcagatactcccgtagaagttctggggtatgcccgtgttggaggccactgggagaccttggcgtcgcgaacaagccacttgaaagagggtcttcagcggcttatatgcctg 30 aggaactgaacgagctgaagattgtgctcagcaagatcctcgatccggaaatgtcgcctctgggctttgatgccaaaaccatgtac aactttgtggccattcgatttgagaagacaacggcaaaggtgcagcagcagcactccactggctgcagatcctcaccaagctgg agattctcattccactggtccagttgttcgccatgttcggcgatggtgttcgcataatgaaatacggcatccagcacgagctgatgcg cgagaaggatgcccaatctcagtccctggccaaggctcccaagaccccgtgtaaagagagcaaggagaccaaagcggatatg gcca at ccgcccaggcctcct gtt gtcgaggat gactct ggtaat acgtct gccatttcggat gacgaggcgcccacgaat cgtcaggat gacgaggcgcccacgaat cgtcaggat gacgaggcgcccacgaat cgtcaggat gacgaggat gacgagagat gacgagat gacgagagat gacgagat gacgagagat gacgagat gacgagagat gacgagat gacgagat gacgagat gacgagat gac35 cacgga at tete cacgga t get gag cae a at ete acct gtt geat cet cat get ggae at act tet ga ag cae at gga act acct get gag cae at ete cac gga act acct get gag cae at get gag cae at ete cac gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag cae at ete cae gga act acct get gag act acct gag act acct gag act acct get gag act acct get gag act acct get gag act acct get gag act acct go gag acct gcgtggagcagcacatgggcatccatacgagtgtctgcgagaacgtctccaggctgatcaagtgcatggtcactgcagctcgagtgggtetcagtagteatgtetgegeettaaaggtteceategaggacateattgaggaagaaaagteetegegeaaateteeaeeeg 40 cagacccttaa

Human Homologue BLASTX with EST: Phosphatidylinositol transfer protein (P48739)

45 Drosophila EST SD01527 (Al530804), GH18602 (Al387906)

Annotated Drosophila genome genomic segment

AE003725

64

Annotated *Drosophila* genome Complete gene candidate CG5269 – vib PIP transfer protein

Human homologue of Complete gene candidate 1e-90 1346772 P48739

PPI2_HUMAN

PHOSPHATIDYLINOSITOL TRANSFER PROTEIN BETA

ISOFORM

10 Putative function phosopholipid transporter involved in lipid metabolism

Confirmation by RNAi Slight reduction of G1 and increase in G2/M peaks

indicating arrest in G2/M

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PCT/GB01/01297 WO 01/72774

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Line ID 418/32

Meiotic defects in testis: cytokinesis defects. Dark bands in eyes, Category

dominant.

? Reversion **Map Position** 69C 5

> G2E Rescue ID

Rescue Sequence

AGCTAAATAACAAACTCATTACTAGTATATTACTGCCGCCGATTTGCAAGCGC ${\tt GTACCGATCCCGATACCAGGCCAATCGCACTCCCCAGTTGTACGTCATCACTT}$ GTGTGTGCGTGTATTTGCAAAAGAGTGTGTGTGTGTATGTGCATATGACTC GTGCGTTTAGCCGACAATTGGAGAAAAAGCATTAGAATCCCAATTGGCTAACT AAACTAAAGTTGGCTTGGCCAAACATAAACAAAAAGTGCGGGCGCAGCGATT 15

AATATNAAAAAGTGCGTGCGAA

Genomic hit, Accession No. AC006589

SD01527 (AI530804), GH18602 (AI387906) Drosophila EST 20

Rest of results same as line 1104/16

Example 4 (Category 1)

Line ID 1285/1

Category Meiotic defects in testis: cytokinesis defects

5 Reversion ?

Map Position 85D1-5

Rescue ID D8E

Rescue Sequence

Genomic hit, Accession No. CSC:AC014256

20 Associated ORF

Genscan ORF1 predicted sequences

>/tmp/aaaaakfaa|GENSCAN_predicted_peptide_1|702_aa MIQRCVVLLWIVCFCDLFLGLLFLKRKRNAHTPPPPPQFTTYRHLLCYCFRNGEIM ANICLSRLSVLEEIVLLLRVPCAFYFVDYYVPCLLSVLSESFLYHDQLKVFNRTK

- 30 SQAAAAVAAQQQHQHPHQQHPQQQQQQQQQQQQQHPHHLMGGGNGLGNGNG
 LGIQHPGQQQQQQQQQQQQQHPGQYNANLLNHAAALGHMSSYAQSGSMYDH
 HGGAMHPGMNGGMPKQQPLGPPGAGGPQDYVYMGGQTTVPMGAAMMPPQNQ
 YMNSSAVAAANRNAAITTSTAKKLWEKSDGKGVSSSTPGGPLHPLQIPGIGDPSS
 VWKDHTWSTQGENILVPPPSRAYAHGGASDTSNSGNAGILSPRDSTCAKVVEYVF
- 35 SGSPTNKDSSLSGLEPHLRNLKFDDNDKSRDDKEKANSPFDTNGLKKDDQVTNSN GVVNGIDDDKGFK

>/tmp/aaaaakfaa|GENSCAN_predicted_CDS_1|2109_bp

gtgccgccgcagccgccgtcggctatgtcttccagcagcgtccatcgcctggtggggttggcgtcggcgtgggcggagtg

67

WO 01/72774 PCT/GB01/01297

ggtggcggtgtgccaggggtcggagccgtaggctcgaccttgcacgaggccgccgccgccgagtacgccgccactttgccc agaagcaacagcagacccgatgggcgtgcggcgacgacggccatgggatcgataacccggacaaatggaagtacaatccgc cgatgaatccggccaatgccgctcctggcggtccaccgggaaatggcagtaatggtgggcccggcgccattggaaccattggc atgggcagcggattgggtggtggtggcggcggcggagctggcggaaataatggcggctctggtacgaatggcggtctgc atcatcaatcgatggccgctgcagctgcgaatatggcagccatgcaacaggcggcggcgttggccaagcacaatcacatgatat cacaggcagcagcagcagctcagcaacaacatcagcatccacaccagcagcatccccagcagcagcaacagca gcaggcgcagaaccaggggcatccacatcaccttatgggcggtggcaatggactgggcaacggcaatggattgggcatacaa cateceggecageaacageageageageaacaacageageageaacateceggecagtacaacgegaatetgettaace atgcggctgccttgggtcacatgtcatcttatgcccaatcgggtggcagcatgtacgaccatcatggtggagccatgcacccggg aatgaacggcggcatgcccaagcaacagccattgggtccacccggagccggaggaccccaggactatgtctacatgggtggc cagaccactgtgcccatgggagccgcaatgatgccgccacagaatcaatatatgaacagctctgctgttgcagctgccaatcgga at g cag c g at tacca cat c cac t g c caa g a a at t g t g g g a g a a at c c g at t g c a a g g cat t c c c g g t g g a cat c cac g g t g a cat c cac g g t g g a cat c cac g g t g g a cat c cac g g a cac g acgttgcatcccctgcagatccccggcatcggggatccctcctccgtgtggaaggatcacacctggtccacacagggcgagaatat attggtgccgccccctcgcgagcctacgcccatggaggcgcctccgatacttcaaacagcggcaatgcgggcatactgagtccccgcgattcgacttgcgccaaagtggttgaatatgttttcagtggctcgcccaccaacaaagatagctcgctttccggattggaaccgcatttgcggaatctaaagtttgacgacaacgataagtcacgcgacgataaggagaaagcaaactctccgtttgacacaaacggtt tgaagaaagacgatcaggtcacaaactcaaatggtgttgtcaacggcattgacgatgacaagggcttcaagtga

Drosophila Gene Hit TBLASTN of ORF1: pumilio protein (L07943)

Human Homologue TBLASTX with pumilio: Soares fetal heart NbHH19W Homo sapiens cDNA clone (W77820)

Annotated *Drosophila* genome genomic segment AE003681 Annotated *Drosophila* genome Complete gene candidate CG9755 – pumilio RNA

25 Human homologue of Complete gene candidate

le-154 1944416 dbj|BAA19665| (D87078) similar to D.melanogaster pumilio protein (S22026)

30

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Putative function

Putative RNA binding protein which is localised to the cytoplasm. Wild-type allele of pum involved in development of the abdomen (embryos) and of the imaginal discs (larvae or pupae), perhaps having a function in signal transport.

Confirmation by RNAi

Only wild type profiles observed

PCT/GB01/01297 WO 01/72774

68

Example 5 (Category 1)

Line ID

Meiotic defects in testis:segregation defect, cytokinesis defect Category

(Ck-09/32)

Reversion NR Map Position 93B4-8

2C9P Rescue ID

10 Rescue Sequence 1

CAAGAAGCAGCAGCAGCAGCAGCAGTAGAAATAGCAAAAAGGAGGCAGCAAC AACAATAAGCTAGAGAAACCGCCAGCAGCAGCCCCCTAATAAAGAGCAGAGA AAAAAATGAGTTCAAGTTGTGAAAGGTGTGTGCCGTTACACTACAAACTACAA CACCACCATCAGCGCAGCAAAGAAATACAACAACAAATACGGCAATCTCCA GACAACGCGAATGTCGAAATTGTGTATACAATTTATTAAGAAAGCAAGAGCA GCAACAACAATGACCAGCTGCAGTTCATCAGCGGTGTCCTCCTGAATGCCGCT GTCGTCGTTGGTGTCTGCCACCGGCGGTTCCTCAATAATAAGGGCAGGAGGAG CTGCTTAGGTGCACACAATGTAGTTTGGCTTGGTGAATGCTTCTCTTTTTGTTG CTGCTGGCGCATACGTTCCTCTCTCCCCTCATGATCTCAGTTGTCTGCATCGA 20 TGAGCCGCCACCAACGGTGGCTTCTTCTGCTCCTCTTTGGCAACGGACTGCTG CAGTCTTGCCAGAATTTTTCCTAAAATACTGAGCTTCAACTTGGTCTGGT AATGGTATACCATAAGCCATGGACTTGATGCCCCTACAAAGCTCTGTGATTTG **AAATGGGATGCA**

25

5

2C9E Rescue ID

AAGTAGTTCTCTTATGGATGCATC

Rescue Sequence 2

CGTTTCGGCCGCGACAGCGAATATGCAATTTTCCTCTCAATTGATTTTTTACA CACTCGCACTCCTTTTCACATGCGTGCAGTTTATGTTGCTATTGCTGCTACTGC TGCTGTTGTTGTTGTTCTGGCTGCCGCTGCAGTGCAACTTGTAACACT TTCACATTTATGACATAATGCACTGGCCATATTTTTGCTTGGCTCTCCGTTTGT TCGAACGCGAGACGATCCGCTTTTCGCTGCATCTATGCGCTGAAGATGTGCTG CAGTCGATGGGCTCGTCGATAGTGGGAAGGCTCGGTGCCGGCACTATCGATTC 35 CCAACACCATACGATAATATCGGCTAAAGTTATCAATATCGAAGTTTACTATA TTTCGGGTTTTTACGTTTTAAATCTACCTTATCAACATTTTTGNAAGAAGTAAA

40 Drosophila EST several including LD10379 (AA816796)

AE003733 Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidate CG3421 - novel protein with weak homology to myosin

69

Human homologue of Complete gene candidate

5

Ensembl predicted Gene:ENSG00000071333

Clone:AC022505

Contig:AC022505.00011 5.60E-37 (predicted protein with Core domain in kinesin

and myosin motors ENSG00000087179)

10 Putative function Possible novel motor protein involved in cytoskeleton organization

Confirmation by RNAi Marked reduction of G1 and G2/M peaks indicating fewer cycling cells

WO 01/72774

70

Example 6 (Category 1)

Line ID 293/9

Mitotic defects in brain: cytokinesis defect Category

(no overcondensation of diploids, very high polyploidy)

Reversion Map Position 66B

2G5E Rescue ID

10 Rescue Sequence

GTACAAACGAATTATTTGTCTCCTTGTGCGTTCGTTTTATTGTGTTTCGAGTTCT GTTGGTGTGTTTTTGTGTATGTTCCACGAGTTGTTCGCATTAAAAAATTAAC TGCAGAAGATCCATGGAAATGGAGACCATTGAAGAGCAATCGAAGTGCGGTG AGTACTGAAAGAGGGCGCGGGGCGTGGCAGCTCCAAATGGCCGGCGAATTTA TCATTTTCAATGTCGTCCAAAGGGGTTGGGTACGGGGTAAAACCACATTCGG

15 GGCCAAAAGATCCTCATAAAAAATGTCGCTGCCAGCAAATGCAAAAAATAAA ATAAAATAAGAACGACTATAAGTACATCTTTGTGTGTATTTGTGTGACTAAAA AAGCAACGGCATCGTGTCGCANATATTTTAATCTTTNTTTCTGAATTTATTTCG

20

5

Genomic hit, Accession No. AC008303

Associated ORF

Genscan ORF1 predicted sequences >20:53:38|GENSCAN predicted peptide 3|261 aa 25 ${\tt MMDNDDALLNNGGPQSGAETVYGTEDNNMVMSEKCRIFPATQRTGFVGATFSG}$ VLLLDLGALQHCDVIRIDVNIATLEQIKRERQEELAARERIRAQIAADRAEQAQRF NTPDISSTTNSVAATAASNVITTDASVSSVDETRLQIRLPGGIQRTKSFPAGEVLAT VRVYVRNEMLAASDVRDFTLATSYPRREFQTEDEVKTLNELNLVPNAVVLVLTK

EQVNPADDQTAKRSASTKRTKTHRHKRQLMADEPQSDHYKN 30

>20:53:38|GENSCAN predicted CDS 3|786 bp

atgatggacaacgatgatgcactgctcaacaatggaggaccacagtccggagctgaaactgtctacggtaccgaggacaacaac atggt catg toggaga agtgccg catattcccggcgactcagcgtactggatttgtgggcgcgacgttttcgggagtgctgcttcttgatcttggtgccctccagcattgtgatgtgatccggattgatgttaacattgcaacgctggaacagattaagcgtgagcgtcaggag35 gagctggcggccagggagcgcattcgtgcccaaattgcagccgatcgggcagagcaggcacaacgttttaatacgccggacat tagcagcacgaccaattcggtggcggccaccgctgcctccaacgtgatcacaacagacgcctcggtgagttcggtggacgaga cgaggctgcagatccgactacccggtggcattcagcgcaccaaatcctttccagccggcgaggtgctggctaccgttcgtgtcta cgtgcgaaacgagatgctggcggcgatgtacgcgactttaccctggctaccagttacccacgaagggagttccaaacgg aggacgaggtcaagaccctgaacgagctaaatctagtgcccaatgcggtggttctggtgctgaccaaggagcaagtgaatccag 40 ctgatgaccagacagcaaaacgatcagcaagcaccaaacgcacaaaaaacacacagacacaaagcggcaattgatggcagacga gccacaatctgaccattataaaaactga

Drosophila Gene Hit rescue sequence: pebble (rho1 GTPase exchange factor)

45 (AF136492)

Human Homologue BLASTX with pebble: KIAA0337 (AB002335)

71

SD09146 (AI542703), SD01796 (AI530981) Drosophila EST Annotated Drosophila genome genomic segment AE003557 Annotated Drosophila genome Complete gene candidate CG8114 - pbl pebble rhol GTPase exchange factor 5 2224615 dbj|BAA20795| Human homologue of Complete gene candidate (AB002335) KIAA0337 [Homo sapiens (3e-21) also mouse homologue 3e-95 10 42359 transforming protein (ect2) - mouse >gi|293332 (L11316) ect2 [Mus musculus] 15 A guanyl-nucleotide exchange factor involved in signal **Putative function** transduction which is localised to the mitotic anaphase. pbl is required for the formation of the contractile ring and the initiation of cytokinesis in Drosophila

Confirmation by RNAi Slightly reduced G1 and G2/M peaks indicating fewer cycling cells

72

Line ID

542/3

Category

Mitotic defects in brain: cytokinesis defect

(very high polyploidy)

Reversion

NR

5 Map Position Rescue ID 66A 2A1E

Rescue Sequence

- 15 ACATCTTAGTGTGTATTTGTGTGACTAAAAAAGCAACGGCATCGTGTCGCANA TATTTTAATCTTTTTTCTGAATTTATTTCGGNGTANAAAATATTTATCGCATA AATGCGAAATGCCTCCCTCTCTTCATCATCGNTTCCCCTNACTCTCCCTCTCTT CGCCCGACACTGTACCGACGCAAGAAGAAC
- 20 Genomic hit, Accession No. CSC:AC018042

Drosophila EST

SD09146 (AI542703), SD01796 (AI530981)

rest of results same as line 293/9

73

Example 7 (Category 1)

Line ID 229/30

Category Mitotic defects in brain: cytokinesis defect. Meiotic defects in

testis: cytokinesis defects

(Mitotic higher level of condensation, polyploidy, Meiotic:

Ck05/07)

Reversion ?
Map Position 91F

10

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Rescue ID A7E

Rescue Sequence

TCTTGGCCAAACAACGCGAGCAGCTGATGTCGCATGGTGGGAAAATGAGGGT
GGCGCGAGTGGAAGTTGCCATATCGCTGCGATCACAAGCAGCAAATATGGAA

15 GATTAAGCGGAAAACGAAAGACAAAATAATTACAATCAAACAACCGAATTAT
AAAAAGAAAATGGTTTGTCCTCCGAGTTCGTTTAAATATGCTTATCTACGTATC
AATTAAAAAAACCGTAGAAAGAAATTCACGATTCACCCTAATCTAGCTAAGA
CACCAACCAAAAATTCCGATTTACTTTCAGTTGAAGTTGTTACACACTTT
TCTTGTCGATGTTTTGAAGCGCCCCATTGAAATTGATCATTTTTCCA

20 AATTACCCACATCCATTACAATAAATTTAAATTGCTTATTATTTGATTTTACT TGGGAAAATCCCGTTGCCAAATTGGAATTACAATTCCAGCTTGGAATCCGTCA AACTTTACAACATAAACTTATTGTTCTTTTCCGGACAATGCTTCCA

Annotated Drosophila genome genomic segment AE003686

25 Annotated Drosophila genome Complete gene candidate CG6284 - novel protein

possible sir2 family of

transcriptional

regulators/telomeric silencing

30 Human homologue of Complete gene candidate gi7706710

0268A424791DE5BF

|ref|NP_057623.1| sir2-related protein type 6 [Homo sapiens]

(1.10E-74)

35

Putative function Putative transcriptional regulator

Confirmation by RNAi Complete loss of G1 and G2/M peaks indicating fewer

40 cycling cells

74

Line ID 1104/16

Category Mitotic defects in brain, Cytokinesis defect (no overcondensation

of diploids, high polyploidy)

Reversion R
5 Map Position 92A

Rescue ID B5E

15 TTTTTGAAATGTGAAAATGTGGGTTACCCCCAATTCTTATTCGAAATTAAATAA CCTAAAGAACAGTTATACACAGATAGGTAATTTGCACATAAGCCAAATTTTGT CTAGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCCGTGATACGCC TATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTA

20 Rescue ID B5P

Rescue Sequence

CTCCGGACACGCAGTAGCTAAATAACAAACTCATTACTAGTATATTACTGCCG CCGATTTGCAAGCGCGTACCGATCCCGATACCAGGCCAATCGCACTCCCCAGT TGTACGTCATCACTTAAGTAATAAATCAGCGGCAAATCGCATAAATTGCTATT

- 30 GCAAGTGCATTTATATTTGGAAATAATAAATGCTACAAT

other results same as 229/30

PCT/GB01/01297

Example 8 (Category 1)

Line ID

WO 01/72774

343/5

Category

Mitotic defects in brain: cytokinesis defect

5

(very high polyploidy, chromosomes entangled?)
NR

Reversion Map Position

75B

Rescue ID

C6E

10 Rescue Sequence

Genomic hit, Accession No. CSC:AC015427

GTTACCCTTATATTAATTTTCAAATTTCTAAATAATCAA

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Associated ORF

Genscan ORF1 predicted sequences

MVCAMQEVAAVQHQQQQQQLQLPQQQQQQQQTTQQQHATTIVLLTGNGGGNL HIVATPQQHQPMHQLHHQHQHQHQHQQQAKSQQLKQQHSALVKLLESAPIKQQ QQTPKQIVYLQQQQQPQRKRLKNEAAIVQQQQQTPATLVKTTTTSNSNSNNTQT TNSISQQQQQHQIVLQHQQPAAAATPKPCADLSAKNDSESGIDEDSPNSDEDCPN ANPAGTSLEDSSYEQYQCPWKKIRYARELKQRELEQQQTTGGSNAQQQVEAKPA AIPTSNIKQLHCDSPFSAQTHKEIANLLRQQSQQQQVVATQQQQQQQQQHQHQQQ QRRDSSDSNCSLMSNSSNSSAGNCCTCNAGDDQQLEEMDEAHDSGCDDELCEQH HQRLDSSQLNYLCQKFDEKLDTALSNSSANTGRNTPAVTANEDADGFFRRSIQQK IQYRPCTKNQQCSILRINRNRCQYCRLKKCIAVGMSRDVLRLEQPKAGAKNKSCE PSKNSTVNQINSKLELGNSNEMK

>21:55:09|GENSCAN predicted CDS_1|1533_bp

Drosophila Gene Hit TBLASTN with ORF1: ecdysone-inducible gene E75B (X51549) and nuclear receptor superfamily protein (U01087) BLASTN with genomic sequence matches ecdysone inducible gene

Annotated Drosophila genome genomic segment AE003522

Annotated Drosophila genome Complete gene candidate CG8127 Eip75B ecdysone-inducible gene E75B nuclear receptor NR1D3

Human homologue of Complete gene candidate

ORPHAN NUCLEAR
RECEPTOR NR1D1 (VERBA RELATED PROTEIN
EAR-1) (REV-ERBAALPHA) Q15304 (9.40E-74)

Putative function Ligand-dependent nuclear receptor, putative transcription factor

Confirmation by RNAi Slightly reduced G1 and G2/M indicating fewer cycling

cells Slightly reduced G1 and G2/M indicating fewer cycling

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77

Line ID 448/23

Category Mitotic defects in brain: cytokinesis defect

(very high polyploidy

Reversion NR 5 Map Position 75B

Rescue ID 2G4E

Rescue Sequence

GCTGGTGGACGCTGCTTTCATTCGCAAATTGCTCGTCGTTGGCAGCGGTTGTGC AGAGCAAGAAAAGCGCGCGAAAAAACCAAGCAAAAAATTAATACAGCTGGAT CAAGCGAAAGAGATAGAGAGCAGAGTCAACAGCAACAAATGTTCAATAGCA AATGATATCGCATATTTTTGTTGGTGCCAGTGAAGTGAGATCAAAGTGAAGTG TGCAATGTTCCTTATTAGCAAATCGTAGAGCAACCAACAATCGAGAGTTCAAG TGTCATTTCGAAGCCAAAAAGCAAAATCTCTAATTCAAATATGGTTTGTGCAA TGCAAGAGGTTGCTGCTGTGCAGCATCAGCAGCAGCAACAGCAACTCCAGTT 15 AACGATAGTGCTGCTGACGGGCAATGGCGGCGGTAATCTGCACATTGTCGCCA CACCGCAACAGCATCAGCCGATGCATCAGCTCCACCATCAGCATCAGCATCAG CATCAGCACCAGCAGCAGGCCAAGAGCCAACAGCTGAAGCAACAACACTCGG CGCTGGTCAAGTTGCTGGAGTCGGCGCCCATÇAAGCAGCAACAGCAGACGCC 20 CAAGCAAATTGGTTACCTGCAGCAGCAGCAGCAGCAACCGCAACGCAAAAGA CTGAAAAACGAAGCACAATCGTACAACAGCAACAACAACACCTGCAACAC

Genomic hit, Accession No. CSC:AC015427
25 Drosophila EST GM03519 (A801874)

Other results same as line 343/5

Example 9 (Category 1)

Line ID 36/1

Meiotic defects in testis: cytokinesis defects Category

(Ck-04/06) `

Reversion 79C Map Position

A8B Rescue ID

10 Rescue Sequence

5

GAGTAAAGTAAACTACAGAGAAAAAACGCTTTACGGCGAGAGAACGCTTTAA AAAACACCGCTTGGGAAAAATCTGTAGGTAGANGAAAGGAGCTCACGTTTTT CTGGTGCAGATCGAAATCGGTATCGGGTTTATTCGCTTTGCCGGATTGTTACTT

CACGTTTGTTAATTGCGTTTCTTTGTTTCTTATTCTCCTGCGCACACTTTGATTT GCGTTTGCAACTCGCAATTCGCAATTGGCATTTGCTATGCGACAACTGCCGTT ATTTCCGGTCCGTTTACTTTTCCAATGGCTTGCCTACACACCGCCAAACTTTGG CTTGAACTTGGGATATTGGTTGCCCGAATTTCCTGANAAATTTTTCCTT

Genomic hit, Accession No. CSC:AC013886 20

Associated ORF

Genscan partial ORF1: >18:33:59|GENSCAN_predicted_peptide_1|99_aa CICFALLGLLIRRKLMVVFGSTSRKAQSLESRRAKNTSQKIGNQYPKFSQVCGKPS KSNDRNNGSCRIANANCELRVANANQSVRRRIRNKETQLTNVK

>18:33:59|GENSCAN predicted CDS 1|300 bp

tgtatctgcttcgccctgcttgggctactcattcggcgaaaattaatggtggtgttcggttctacgtcgcgcaaggcacagtctctaga gtctcgcagagctaagaatacatctcagaaaatcggcaaccaatatcccaagttcagccaagtttgcggcaagccatcgaaaagt aacgaccgaaataacggcagttgtcgcatagcaaatgccaattgcgaattgcgagttgcaaacgcaaatcaaagtgtgcgcagg agaataagaaacaaagaaacgcaattaacaaacgtgaagtaa

Drosophila Gene Hit rescue sequence and TBLASTN with ORF1: nucleic acid binding

protein (mub) (X99340)

BLASTX with nucleic acid binding protein: poly(rC)-binding 35 Human Homologue

protein 2 (hnRNP-E1) (S42471)

several including LD32520 (AA951799 BLASTN matches nucleic Drosophila EST

acid binding protein (X99340)

Annotated Drosophila genome genomic segment AE003596 40

Annotated Drosophila genome Complete gene candidate CG7437 - mub mushroom bodies RNA binding protein

Human homologue of Complete gene candidate 4826886

ref[NP 005007.1|pPCBP2] poly(rC)-binding protein 2

45

25

79

>gi|542853|pir||\$42471 (4e-75)

5 Putative function A putative RNA-binding protein specifically expressed in the CNS of Drosophila melanogaster

Confirmation by RNAi Only wild type profiles observed

80

Line ID 472/22
Category Female sterile

(anaphase bridges, lagging chromosomes)

Reversion ?
Map Position nd

Rescue ID sau 5'spl

Rescue Sequence

10 GCACGATCNCTAAAGTCTNGCANAGCTAAAAATACATCTNAGAAAATCGGCA ACCAATATCCCAAGTTCAGCCAAGTTTGCGGTGTGTAGGCAAGCCATCGAAAA GTAACGACCGAAATAACGGCAGTTGTCGCATAGCAAATGCCAATTGCGAATT GCGAGTTGCAAACGCAAATCAAAGTGTGCGCAGGAGAATAAGAAACAAAGA AACGCAATTAACAAACGTGAAGTAACAATCCGGCAAAGCGAATAAACCCGAT

15 ACCGATTTCGATCGGTGCGGGCCTCTTCGNTATTACGCCAGNTGGCGAAAGGG GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGGCC

ANTGCCAAGCTCTGCTCTAAACGACGCATTTCGTACTCCAAAGTACGAAT TTTTTCCCTCAAGCTCTTATTTTCATTAAACAATGAACAGGACCTAACGCCNGT

20 AAC

5

Rescue ID Sau 5'splac

Rescue sequence

25 GTTGTGATCNTCTTGGTNAATCNNNTTGGAAATTCCCCTAANGCTTCCGACAA
TGACCNGNCNTACNNAGCAAANAATNGNAGNACNNGCNGNTGGNCGTANT
ANCAANAACAGGCCCGCACCGATCGAAATNGGNATCGGNTTTATTCGCTTTGC
CGGATTGTTACTTCACGTTNGTTAATTGCGTTTCTTTGTTTCTTATTCTCCTGCG
CACACTTTGATTGCGTTTGCAACTCGCAATTCGCAATTGGCATTTGCTATGCGA

30 CAACTGCCGTTATTTCGGTCGTTACTTTTCGATGGCTTGCCTACACACCGCAAA CTTGGCTGAACTTGGGATATTGGTTGCCGATTTTCTGAGATGTATTCTTAGCTC TGCGAGACTCTAGAGACTGTGC

Other results same as line 36/1

PCT/GB01/01297 WO 01/72774

81

Example 10 (Category 1)

45

Line ID 459/2 Mitotic defects in brain: cytokinesis defect. Meiotic defects in Category testis: cytokinesis defects: 5 (mitotic: high polyploidy, no diploids, higher mitotic index, meiotic: Ck-01/05) Reversion NR 66B1-6 Map Position 10 2D5P Rescue ID Rescue Sequence GCTCCGTTCGAAAGTTGAGAGAGACTTGAAACATATGTTCGGCGTTGCTAGAG CTGGTCGGCTACCGATAGAAACATCGATAGGTCCGATGTTTTTTACTCGTATAT TGATTCANAGTTTGGCTATCGATGTTTTTAGAGTGCCCGCACATTATCTATTTT 15 CATCTCTATTTCGTTGGTATTTTTGTATTTTATGACATTTCGACTGCAAAAGC AGGATGGCAACGCCAGATTGCCGCGAAAGTACGTTATTTTAAATTGGCGCAT TATTATTTAGCTTGTATCATACGAAGTGCACATTACAGCTACGCATCTGAAAT AATAATTTTAATATCGTCTTTTCTCCCATCGATAGAGTTCCGCGCCTATCGA 20 TATATCGTTGATCACCAAATAAATAAAACTAAATAACGCCGCAATGGAACAC GCGACGAGTGAATTGAGGGAATTTATCTCAGATCTTGTAATTCCGCACCACGT TGCAATGGTAACATCAATCCGGATCACATCACAATGCTGGAAGGCACCCAGA TCCAGAACAG 25 AE003557 Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidate CG8038 - novel gene ribonuclease P homology CG7892 nmo - protein 30 serine/threonine kinase involved in eye morphogenesis CG8038- 5e-24 4309676 Human homologue of Complete gene candidate 35 gb|AAD00893| (AF001176) ribonuclease P protein subunit p29 [Homo sapiens] CG7892- protein kinase 40 mitogen-activated 7 (MAP kinase)' gi:4506093

> and gi7706445 D919050533B3C33A |ref|NP 057315.1| nemo-like

82

kinase [Homo sapiens] (3.30E-174)

5 Putative function CG8038: tRNA processing enzyme Ribonuclease P protein subunit CG7892: a protein serine/threonine kinase involved in cell cycle, possibly targeted to cytoskeleton

Confirmation by RNAi Both showed a marked increase in G1 peak indicating arrest in G1

Example 11 (Category 1)

623/8 Line ID

Meiotic defects in testis: cytokinesis defects 5 Category

Reversion 37E1-3 Map Position

2E2E Rescue ID

10 Rescue Sequence

CTACGGGCATTCGCATGTTCGAACATCTGGTGTAAACAAGTTCTGAGCAGTGT TGCCAACTCTTCAGTTAAACAGTTAAAAATAGCTAAAAAATGTTGACGGTAGC TAAATTATAAAGCTAGAAAAGAAATGATATATGATAAAATAAGTATTTCGACT CACAGCATTTATTATTTAAGACGGTCAGATGAAGTTACAAAAATCCTAAATTG GCCCGCTGTATCTAAGAATTAATACCAAGAAGTTGTCATCAAAGGTCGAACTC GACGGAAATTCTACTTTGAGTTTTTAAATTTAATAAATATGTATTTAAAATTAT TAGTTTTAAAAATGGCCAGATCAAAGACTTTTGAGATATGATACTAATCAAAA GTCGAATTCGCGGAATTAATTCTTGAAGACGAAAGGCCTCGTGATCGCCTATT TITATAGGTAATGTCATGATAATAATGGTTTCTTAGACGCAGGTGGACTTTTCG

20 GGGAAATGTGCGCGGAACCCCTATTTGTTATTTTCTAAATACATTCAAATATGT ATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG AAGAGTATGAGTATTCAACATTTCCGGGCGCCCTTATTCCTTTTTTGGGCGCCAT

25 **CAGT**

35

15

AE003662 Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidate CG17559 dnt - doughnut protein tyrosine kinase

30 Human homologue of Complete gene candidate

Homo sapiens RYKreceptor tyrosine kinase GDB:21773

Putative function

growth factor transmembrane receptor protein tyrosine kinase

involved in cell growth and maintenance

Confirmation by RNAi

Only wild type profiles observed

Example 12 (Category 1)

Line ID

629/14

Category

Meiotic defects in testis: cytokinesis defects

(Ck-06/09)

Reversion

5

NR 64D

Map Position

Rescue ID

2A9X

10 Rescue Sequence 1

GACGGGAGGAAGTAAGTGGGAGGAGAGAGTAGTGCCTCTTTTTTACTGGAGA AATGGACAAACTCTGGGAACTGCGAACTGCGAACTAACCGAGGCAAAAATTG AGAAGCGAGCTGAAAGCGGAATTCAAACAACGCAGCGCTGACGCGACGCCG GCTAATGAATGAACGAGGCGGAATGCGGGAAGAGCGCAGAGAGGCGC 15 AATGACAAAATAGTTGTAGAAAAGCGCCGGCAAGCGGAACTCCACACTCTTT CTCACTCTCTTTCCACCCACACCCCTAGTTCACCGGAAAAAGAAAATTCGTT TGCGGCGGGGTGTATTTTTCACCAAAAAGAGAGTGTGTGCAAAACGCTAGA GAGAGAGAGAGAGAAAGAACTGACGTCAGTTCTGCCTCCGTCGACGCC GCTGCCGGCGTCCCAAAGCGCCACCACCAAAAAAAACGCGAGAAGAAGCAGA 20 ACAAACACACAAAAATTCGCACAGTGGAGCAGAAATCAAGC

2A9E Rescue ID

Rescue Sequence 2

CTCCCGTCGTTTTGAGATCAGCTGCTCTCGCAACAACAACAACTATAACTGTA 25 GTTACCGTCTCTTTTGCATCGTTCGTTTTTCGTTTGTGTCGCCAAGTGATTGTGT GTGTGCGTAAGCTTAAAGCTGACTAACAAAACGAAACAAGAAAAAATATAAA TTATAGGAAAATTGTTAAATTATAACCAGAAAGAGAGCGGCACTTACGTGTGT TATTGTGTGCGTGTGCTTTAAAAAGATATAAAAAATAGCAATAGAAAGTTATTA AAGCGTTGGCAAAAAAGTCCAACGAACAGCGAGAGGAAGCGGAGAACGAAA 30

TAGTTAAAGCCAAAGTCGCTGCCGACGTCGCACTTGAAAACGTCGCAAAAGTT TGTAAACACACCAGTGTGTGTTCGTGTGTTTTTTGCCGGCGTGCCAGTGTGCG TGCGCCTAGAAAAGAGTAAAGAAGCAGAAGAAAAGGAAGAAGCCGAAGAAG CAGCAAAAGAAGCCGACAGCAAAAAGTAAATAAAATCAAATGCCCCCTGGCA

GAATAATATTAAATTAAGACACATACTCAAATTAATAAC 35

Genomic hit, Accession No. CSC:AC015076

Drosophila EST

LP08767 (AI295205)

40

AE003567 Annotated Drosophila genome genomic segment

Annotated Drosophila genome Complete gene candidate CG10668 - novel with

homology to ssDNA/RNA

binding proteins

Human homologue of Complete gene candidate 45

CG10668 - 3e-12 4506449

85

ref|NP_002889.1|pRBMS2| RNA binding motif, single stranded interacting protein 2 >gi|1082

5

Putative function Possible single stranded DNA/RNA binding protein

Confirmation by RNAi Slightly increased G1 and reduced G2/M indicating G1 arrest

86

Example 13 (Category 1)

Line ID 653/12

Category Meiotic defects in testis: segregation defects, cytokinesis defect

(Ck-07/35)

Reversion NR Map Position 75B

Rescue ID I5E

10 Rescue Sequence

5

15

20 AAACTTTCTTGGGGAAAGTGAAAGCCACGTATCAGACCAAAATCCACCCAAC CCTGCACACACGCATCCCCATAAAGAACGACCTTGAGCT

Genomic hit, Accession No. CSC:AC014071

25 Associated ORF

Genscan ORF1 predicted sequences >16:36:33|GENSCAN_predicted_peptide_2|477_aa MLILMRPSIKLAANQNAIKAPNGPKNFLDKVLVVRCWLSVCLLENGHIAVTASGS NNNNNSNNINLNLKANYQMSATSIRDSFATILLDAQNRVQNATVAAKNFMLPLR LRSDTSGDTSNNNENNSRRARQAYNCGVNWLTTHRPKRRRQVHPPLGSTPSCNN

- 30 NSSKISRNSSSSSNNIASATATRIFLGTSAILAIDFDNTRVPGYYQPTGEWIWVSKS MIKQLFAVAATADDVAAAAASRGNALTFLPGKEKGPRKKAEGCGMEWSGVEWS GGDVMCVLSSVATVDDDDHHGGGHFDGLLGTPSALIRLNCLINPKKMRMDFEVE VAWQIARAADLRLISMHLNVPYEMKTMKTMESVIDGGSLYQPTALFGSLFCLVY SSAADVLLLLANCKSLAHGVDVDCDSDASRGSDCDVGHFSPSFRCRFQLSLVAQS
- 35 ARHANALKSQVTTATSSSSNNSDSLANKQTNQHIFVYQLSA

>16:36:33|GENSCAN_predicted_CDS_2|1434_bp

25

10 Drosophila Gene Hit rescue sequence, ORF1 and genomic sequence: Canton S E78B nuclear receptor superfamily protein (U01088)

Drosophila EST LP11082 (AI296953 similar by BLASTN to U01088)

Annotated *Drosophila* genome genomic segment AE003593 Annotated *Drosophila* genome Complete gene candidate CG18023 - Eip78C

Ecdysone-induced protein 78C nuclear receptor NR1E1

Human homologue of Complete gene candidate

CG18023- 4e-32 119100
P20393 EAR1_HUMAN V-

ERBA RELATED PROTEIN
EAR-1
Scill 082832|pir|| 4 32608

>gi|1082832|pir||A32608

Putative function ligand-dependent nuclear receptor, putative transcription factor

Confirmation by RNAi Not done due to failure of PCR procedure

88

Example 14 (Category 1)

Line ID 876/2

Meiotic defects in testis: cytokinesis defects Category

Reversion Map Position 73A

> Rescue ID 2H1E

Rescue Sequence

GATCAAACAGAAAATCCAAAAACGAACAGCGCGCGCGAACGAGAGCCGTT GAAGCCGCCAGAGAAGTGCGCTGCTCGCGTCGCTGCCGGTATGTGCGTGTCTG TGCACTGAGAGAAAATGCTCGATTAAACAGAGAAATTAATAGTAATATAAAA AAAAAAAAATTTGTTTATTATTCTCAATTCAATAAAATGTAATTATTATTAT ATGTACATCTAACAAAAAATGTTATTATCTTATAACAAAGAGGTAAAATCATA 15 AGTAGTACGAAATCTTTAAAAGAGAAAGTGTGTTACGCAAAAAGTATTCAAA TGTACATTTCATTAAAGCTAATGGTATAATTAGGTATTTACAGTGTTTAGCTAA GGCTTTCATCTGAAATATTTATTAATTATGTCTAGTTGACCTGTTTTTAGTTTTT

TTGNATAACAATATTTATTATTTATTAAGGAAAACAAGGGGAGAAGAAAAAC 20 CTTAATTGAAGCAAAGCAGTCTTTTGAACCCACTGGTG

Genomic hit, Accession No. AC005633

Drosophila Gene Hit rescue sequence: argos (M91381

25

Annotated Drosophila genome genomic segment AE003527 Annotated Drosophila genome Complete gene candidate CG10162 - Egf2 translation facto

30 Human homologue of Complete gene candidate CG10162 - 4e-11 181969 (M19997) elongation factor 2

[Homo sapiens]

Translation elongation factor Putative function

35

Confirmation by RNAi Not done due to failure of PCR procedure

CATEGORY 2: FAILURE TO ENTER M-PHASE

Example 15 (Category 2)

1216/12 Line ID

Category Meiotic defects in testis: no division

(no meiosis)

NR Reversion Map Position 82F1-2

2I5X-1 Rescue ID

Rescue Sequence 1

AAACCAAGCAACAGAAATATCTCCAGTAGAGAGCGCCACTGGAAGATCGGAA TTTTTAGTGCTCTGCTCTGACTAACAGGTTTTAGTAGTAGTGCTTACTTTTCTAC TACGATITTTGTCGCGGCTAACAATTCTGTTTTCCCACTCCCTCTCTCAGTTTTT

- GCATGGTAACTTTTCGGTCATTGTACTGTTGTTGTTGTCTTGCACACCGCAAGA GAACAACAACCAATCGGAGAAACACTGATAGCGCGGTACAGTGGGGCAGGCCA AACTAGÁACCTATACATTTAAGATGTCTCCAATTTGTGATTTTGCCTTTCAAGC ATACTAGTTCATAGTTGATTGTTTTGTTATGTTTTGTCTTGAATGCGATGTTTCA AGAAATCTTATTTTCGAATTACGATATTATTCTTATTCCTTTGACTTATTAAAA
- TAAATGAAAACGGCGAGTAGAGCAAAAGAGCGACCACTGTGGCTCCACAAGC 20 CACCTCACTGCTTGCGACTGCAAATTTGTGCAGCTGAACTTTG

2I5E-1 Rescue ID

Rescue Sequence 2 25

ACAGGTATAACTGTTTGGCGTGAGGGAGCACGAAACTCCAGTGGAGACTTCTC CGCATCGCCAGCGAAACAAACGATCAAAATGAATACTCTGATAACGTGTGAA GGTGAGCAACAAATAAAGTATAAGAAAATACCGCCACGAAAACAACAACA

- ATAGAAATGTCGACGCACCCTTTTCTTTTTCTCGCAAAGAACGAGGAAATGGA GAAGCGCAAAACCACATCCCGCTTAAAGAGTCCCTTTCCCCCGCTGGAAGTGG AAGGAAAGGCAGCTTAAAGAGGAGCGGGTGGCTTCCAGTATGTGGAAAACAA AGCAGACGCCATTGGAATGCCGTCGTTTTTTGTTGTTGCTAAGCCGGACATGG CAATTGTTGCTTTTCGAGAGGGGGGTGGTGAAACTCATAAATATCAGCT
- ATGGCGAGGGGGGGGGGGCAGTCTTTGTCTGACGTACCGTACTTTTAATTTCTT 35 GTCGCCCGGTTTAATCCAATTTATCCAGCTTTGAATTTCGCGG

Genomic hit, Accession No. AC007532

AE003603 Annotated Drosophila genome genomic segment 40 Annotated Drosophila genome Complete gene candidate CG1116 - novel

Human homologue of Complete gene candidate 2495728 HYPOTHETICAL PROTEIN KIAA0258(aa)

90

Putative function No homologies which indicate function

Confirmation by RNAi Slight loss of G1 peak

91

Example 16 (Category 2)

Line ID

1344/15

Category

Mitotic defects in brain: no mitosis

5 Reversion

NR

Map Position

83C

Rescue ID

2F6E

Rescue Sequence

- GGTTATCACACATCTGGTCCGAGCTATCCAGGCAATCACATTTTTGAAGTTCCGCG GGTTATCACACATATATCGATCATACCCCGAAATGTGTAACACAGATACAGCT CACCATCCCTCTGATAAGATCTTATCAAGTTCGGGCTTGCTCGCTATCGTGAAT TGGGTTGAAGGGTCCGCGATAATTGCATTGGGCATGCCATTGGTAATCACAAT TGGCTGATAATGCTGCTGCTGCAATTCCACGGGTATGAA

25 TTCATCAATTGGTTA

Annotated *Drosophila* genome genomic segment AE003602

Annotated *Drosophila* genome Complete gene candidate CG1347 - novel protein with

:CG1347 - novel prote myosin homology

30 Human homologue of Complete gene candidate

1503990 |dbj|BAA13194| (D86958) KIAA0203 similar to mouse CC1.(aa)

35

Putative function similar to coiled coil protein with ubiquitin like domain

Confirmation by RNAi Marked reduction of G1 and G2/M indicating fewer cycling cells

92

Example 17 (Category 2)

Line ID

703/16

Category

Meiotic defects in testis: segregation defects, meiotic failure

(Mf-07/75)

5 Reversion

Ŕ

Map Position

Rescue ID

2E7E

83B

Rescue Sequence

10 AAGCAGCCCAACAGCTACGCAAAAAGTTACTTATATTCGCAGCAAAACAGAT TITTTTGTTTTAATCGTAAGTATAGGAGTGAAAAATAGCGCTAGAGTAGACCT AAGTACACAGAAAGACAAATAGGGCGAGTAAAATCGCGGTCCTGGTCATTTC TCTGGCCTTGACCAATCCTTTGTCTGCGCTTTCGTTGGAAAAGGGGTTATGTAC GAACTGCGTGCGTACCTAAGGCCAGATTAGTCATCGGGCAGTCATATATTCAT

15 GCAAAAATCATTTGGTGGCCGTCGGCCTTTGTTCGACTGTACCTTGCTCATTA
TTTAATAAGCGCGACAGCAATATACACACTTTGAACCCCCATCCCACATTTTTT
CTCACCGTTTCCCCCTAATTTTCGTTTTCCCTGTGCCCATCATTCCGCTTTCGCC
ATGTCAGTGTATCGCTTCAAAATGGCGCCGAACCACATGTCTTCGTTCTCGGC
TCGTCCGCTTCGTTCGTGCGCTCGTGTGTCTCATTCGCTCTCCGAATTTCG

20 TTTAACAAAGTGGTGCGAGCAGAGGGGCCGCTGGATTCGAGGCAAACAACAC ATATACCTA

Genomic hit, Accession No. CSC:AC013960

25 *Drosophila* EST several including LD15903 (AA440858), GH20091 (AI389018).

Annotated *Drosophila* genome genomic segment AE003602 Annotated *Drosophila* genome Complete gene candidate CG2922 – novel

30 Human homologue of Complete gene candidate 286001 dbj[BAA02795] (D13630)

KIAA0005 [Homo sapiens] also NP_054757.1| HSPC028 protein

[Homo sapiens] e-179

35 Putative function Weakly similar to a region of human and murine

EIF4G2 translation initiation factors; may act as a

translation initiation factor

Confirmation by RNAi Only wild type profiles observed

Example 18 (Category 2)

Line ID

741/3

Category

Meiotic defects in testis: segregation defects, meiotic failure

(Mf-05/31)

Reversion

5

15

NR

Map Position

88D

Rescue ID

H6E

10 Rescue Sequence

GCCTGGAGCCACCTCTAGAGCCACGGCCAAAAAATTGTGTGCCAAAAAATCG
TATGGCGTTACGCATCTTGTTATTCTAGTGTCTTTGGTTCTACAAATCTGGCCA
ATGGGATGGACGGATTTTGGGGCTTTTGCGCCCCACATATGTNTCTTACAACC
CACTCGGCCCGGCAAGTGGGTGTCAATTACGGACATCGGCAATCCGAAGACC
GGAGACCCAGAGACCCTCAGACCCCAGGGCCCCATTCGATTCGATTTCGAGTT
GCGTGGGCCGATCTCACATTAGTCACATCGAAGGAATGAAATAAAAAGAAAA
AACATGACGGCCGAAAAGAACTTATCCATCTTCAAAGCTCTCAGAAAATACA
AAAACTAAAAAACTTTTGACTCTTCGTCTTTCACATTTTGCGCCATGAAC

20 ACGCCGACTG

Annotated *Drosophila* genome genomic segment AE003705

Annotated *Drosophila* genome Complete gene candidate CG12600 - novel protein

25 Human homologue of Complete gene candidate

CG12600- 5e-27 4240227 dbj|BAA74892.1| (AB020676) KIAA0869 protein [Homo sapiens]

30 Putative function

putative cytoskeletal structural protein

Confirmation by RNAi

Reduction of G1 and G2/M peaks indicating fewer cycling

cells

94

Example 19 (Category 2)

Line ID

773/1

Category

Meiotic defects in testis: cytokinesis defects, meiotic failure

(Mf-02/15)

Reversion

5

R?

Map Position

83F

Rescue ID

2D9P

10 Rescue Sequence

20 GGGTTAATCATTTTCTTGCTCCATCTGCTTTTCCCAACTGTATCCAAGTACAAC TACAGCATTATCCTCAACTG

Annotated *Drosophila* genome genomic segment

AE003675

Annotated Drosophila genome Complete gene candidate CG10272 - novel protein

Human homologue of Complete gene candidate

CG10272 - 2995577

AC004490 (AC004490)

R29381_1(aa) protein includes HMG-I and HMG-Y DNA-

binding domain (A+T-hook) found in HMG non-histone components in chromatin

30

25

Putative function

Chromosomal protein

35 Carefin

Confirmation by RNAi Loss of G1 peak indicating arrest in G2/M

95

CATEGORY 3: METAPHASE ARREST

Example 20 (Category 3)

5

Line ID 1067/13

Category Mitotic defects in brain: prometaphase arrest

(overcondensation, polyploidy, scattered chromosomes with

bipolar spindle)

10 Reversion NR Map Position 69C4-10

Rescue ID 2F8E

Rescue Sequence

- 20 TTAATATTAAATCACATATATTTAAGCCTCTTTATATATGTAAATATTTTAA TTTTATTAAAATAAATTATATATTGTTTTGTAATATGATCGAGGGCTGCCACCT TGTGATAAATGCTTACCAACACTTTTAGGTACGCCGTTTAGTGTACGTAAGTTG CGTACCTAGATATCCAGCGAAATCAAAACATTGAGTAAATCGTGGAAAATGG ATGAAAATAGCTTAATCTACGGACTCGAACTGCAGGCGCGGGCTTTAACACCT
- 25 CAGTACGGAGAGCAACGATGTGTGCTTCTTCATAGCCACCAACTCCTTGAA GCCCACCAATCAGGTTCACTTAATCCAGTACGAAGA

Genomic hit, Accession No. CSC:AC020333

30 Associated ORF

Genscan: ORF1 predicted sequences: >16:51:11|GENSCAN_predicted_peptide_2|178_aa MAQNISPEQSGGAGGGGSKHSDDSMPVKDNHAVSKRLHKELMNLMMANERGIS AFPDGENIFKWVGTIAGPRNTVYSGQTYRLSLDFPNSYPYAAPVVKFLTSCFHPNV DLQGAICLDILKDKWSALYDVRTILLSIQSLLGEPNNESPLNAQAAMMWNDQKEY

35 KKYLDAFYEKHKDT

>16:51:11|GENSCAN predicted CDS 2|537 bp

96

Drosophila Gene Hit TBLASTX with ORF1: poor homology to several sequences

including homolog of RAD6 (DHR6) (M63792), bendless

(L20126) and Ubc D1 mRNA for ubiquitin-conjugating enzyme (

X62575).

5 Human Homologue TBLASTX with ORF1: ubiquitin carrier protein E2-C (UBCH10)

(NM_007019.1) and ubiquitin-conjugating enzyme E2B (RAD6

homolog) (NM_003337.1).

Annotated *Drosophila* genome genomic segment AE003541

10 Annotated Drosophila genome Complete gene candidate CG10682 - vihar ubiquitin-

conjugating enzyme

Human homologue of Complete gene candidate gi5902146

0B6F58A1F0665D9A

15 |ref|NP_008950.1| ubiquitin carrier protein E2-C [Homo

sapiens] (2.50E-50)

20 Putative function Cyclin specific ubiquitin conjugating enzyme

Confirmation by RNAi Complete loss of G1 and G2/M peaks indicating fewer

cycling cells. Immunostaining shows metaphase arrest with

condensed chromosomes

97

Line ID 1105/1

Category Male sterile, Female sterile, Mitotic defects in brain: prometaphase

arrest

(Overcondensation, polyploidy, fewer anaphases, high mitotic

index, scattered chromosomes with bipolar spindle)

Reversion R Map Position 69C

Rescue ID A5B

10 Rescue Sequence

5

GTACATATAATCACAATTGAGAATCGAAAACCCGACCGCCACGAAGCGCGCT
AAATTACACGCACATACTGAAAGCCAAACAGCGGATAGCACTAGCATCCTAC
ATATATAGACGTAGATATATAGTCATGGCGCAGAATATCAGCCCCGAGCAAA
GTGGTGGAGCAGGCGGCGGCGGCAGCAAGCACAGCGATGACTCCATGCCCGT
GAAAGACAATCACGCCGTGGAGCAAAAGGTGAGTATCACATGGTGCAGCCTA
AGATAATCCGCCAATATACACACACACTCACACTCACCCACAGACTGCACAA
GGGAACTGATGAACCTGAATGAATGGGCCCACCGAAAAAAGGGG

Rescue ID A5E

20 Rescue Sequence 2

- 30 AAA

Genomic hit, Accession No. AC007328 69B-69C

Associated ORF

35 Genscan: ORF1 predicted sequences

>/tmp/aaaaanjda|GENSCAN predicted_peptide_1|357_aa

MGKKAKHKKGKGPEKTAMKADKKQAARQKKMLEKLGEANIADIIQLLEAKEG KIEAISESVCPPPTPRSNFTLVCHPEKEELIMFGGELYTGTKTTVYNDLFFYNTKTV EWRQLKSPSGPTPRSGHQMVAVASNGGELWFPNFACISRNQSWFVFHNCRLKAA

- 40 SREKVLLNFNGTVLHPANNIIVHVKLFKKANGFKPWLLDVKLDACRFVRTNFHPF VRIIFDLFKDFSTINHTCPYVVLRSMRYIVRRSPRLVHPIVDVPAIGHTRPRRKAAV RGIGCAHRCPLIRMATPCRTNVVMMTLMRGSVRSRVMAICCYRRPAIAIARRRHP TAIAHSQEVAERLGGLLYPDIQRTNP
- 45 >/tmp/aaaaanjda|GENSCAN_predicted_CDS_1|1074_bp atgggcaaaaaggccaaacacaagaagaggcaaaagggccaggaaaaacggccatgaaagcggacaaaaagcaggcgg cgcggcaaaagaaaatgctggaaaaactgggagaagcaaatatagctgatatcatccaattgctggaggccaaggagggcaag attgaagccatcagtgaatccgtttgcccgccaccaactccacgatccaatttcaccttagtttgccatccggaaaaggaggagctc

98

Drosophila EST several ESTs including LD04777 (AA201675)

All other entries as for 1067/13.

Example 21 (Category 3)

Line ID 1407/13

Category Mitotic defects in brain:

(weak overcondensation, metaphase with bipolar spindle)

Reversion NR Map Position 92B1-3

Rescue ID 2D3P

10 Rescue Sequence 1

5

40

- 20 CACATTGTGCTGATGCAAATAAAATTCCAATTAAACGCCCCTGAATGGGAAGA TGACGCATCTTTAATGGGAATATTATGGTAAATTTAATA

Rescue ID 2D3E

Rescue Sequence 2

- TNCGTGATTATCAGCGTTAATTGTACAATATTATGATTTATTCGAGCTGTAAAT
 CTTCACAGCAAGCACAAACTGTAATTATACCACTTAGAATTCCGCGGAATTAA
 TTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTAATGTCAT
 GATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCG
 GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
- GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCT GTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTG GGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAG
- 35 *Drosophila* EST LD05707 (AA246767)

Annotated Drosophila genome genomic segment AE003727

Annotated *Drosophila* genome Complete gene candidate CG7444 - very short ORF with EF hand homology

Human homologue of Complete gene candidate none

45 Putative function Possible calcium binding protein

100

Confirmation by RNAi Slight loss of G1 peak

PCT/GB01/01297 WO 01/72774

101

Example 22 (Category 3)

Line ID

1439/7

Category

5

Mitotic defects in brain: prometaphase arrest.

(overcondensation, polyploid, no anaphases, scattered

chromosomes with bipolar spindles)

Reversion

Map Position

96F10-14

10 Rescue ID G3X

Rescue Sequence

GTCGGATGTAGAAGACGTGCCCGAAACCCAGTTAGAAATCGATGTCAGCGAT GGCGCCGGACTGGAGGATGAGGATGACGATATGGAACAGATTACAGCTC AGAAGGTAAGGTAAATCGTAACAGAGCTTTTTAATACGCAAGTAATCACATTC

TGATATCCCTAGGTTCTGGAAATCATAGAAACCGCGTGGATAAATGAAATGTG 15 TGCGCCGGAGATCCTGCCCAGCCAGACGGACATGCTGGAGCTGATGGTCTCCC AGGTGGCCCATATGGAGGAGCAGATGCGCGATCTGGACAAGAACGATTTCCG AGCGGTGGTGCACTCCATGGAACTGGAGAGGGTGCGCTACATAATGGCCAGT TATCTGCGTTGCCGCCTGCAGAAGATCGAAACCTTCACGCAGCACATCCTCAA

20 AAGTTCGCCCAGGAGTTTGCCAGTAAT

Genomic hit, Accession No. AC007825

AE003754 Annotated Drosophila genome genomic segment 25 Annotated Drosophila genome Complete gene candidate CG14549 - novel

Human homologue of Complete gene candidate

none

30

no homologies which indicate function **Putative function**

Only wild type profile observed Confirmation by RNAi

Example 23 (Category 3)

Line ID 1466/4

Category Mitotic defects in brain: metaphase arrest.

(overcondensation, no polyploidy, fewer anaphases, metaphase

with bipolar spindle)

Reversion NR Map Position 72F

Rescue ID E5E

10 Rescue Sequence 1

5

GGCTGGATGCGATTCGCTTTCGGATTCGGATGCATTCAGCCGCTGTCTCGACA CCGCCGCAACCGCTCTCGGGAGTTTGAAAATTTGAAATGAGCGGATTCGCGTT GCGAAGGCGAGCTAGCGTTGCAGGCAGTGTGGCCAGATGCCGCGTGCGAACG TATTCTCGAATGCAATCGGCCGAGTGCAGATGCACTAAAAATAACCCACTTCC

- 15 AGTGACTGGAAATTAAGATCAAGGNAATAGATTTTATAAAAACTTATATGAGT AAAAATTTTAAAAATTGTGGAGTCAACCTAAATTATAAGCAACTAATTTATAAC ACAAGTAAAGAATGATATTAAGTAACTTTTTAAATAATATTCCATTATGCTTA CGCTCAATTTATGAACAAATGTTTTCTCGATCCTTAGGTAAAGTTTCGAGTTTC GCGACTANATTTATTAAAAATTAAGAACATCTCCATTTATGTACACATTTAAAAG
- 20 ATTTATGAGCGGTAATATTAGCTGGTTGAC

Rescue ID E5P

Rescue Sequence 2

AAACGCCCGCAGTGGCGGCGGGAAAAATCAGAGGAGCCGGAAAAGTCAG CGGCCCGCCAGCGGACTCAGCGGCCGCTCCAGCTGCCGCCCCCGCAGTGGA GAAGGCTGAGGATGCCGATGGCGAAAAAAAGGACGGCGAGGCCGGAAAGCA GGACAAGCAGCAGGATGGC

35 Genomic hit, Accession No. CSC:AC020154

Associated ORF

Genscan ORF: ORF2 predicted sequences

>21:06:03|GENSCAN_predicted_peptide_5|415_aa

- 40 MASEVAQIPAEETPAVAAAEKSEEPEKSAAPPADSAAAPAAAPAVEKAEDADGE KKDGEAGKQDKQQDGEEPKKDEAVAAPVATKSEAPPAQKFNVHKTNFEKDIIYL YQFSRTPLLPSLSPYCLKVETWLRLVGLKYENVDHKMRFRSKKGQLPFIELNGEEI ADSAIIIKELSSKYEKYLDSGLTAEQRNVSYATIAMLENHLIWIIFYWRAKYPDNVLKGYKVNLQHALGLRLPNSILNFFFKITFGRKGTKKLKAHGIGVHSAEEIEEFGKD
- 45 DLKVLSEMLDCKPFFFGDEPTTLDVVAFAVLSQLHYLSKDIAYPLRDYMTEKCPN LIGHVSRMKDKCFPDWDEICTKLDLNAHIPKPEPETKEGKEGGEQEKSNEQEGTE

103

GDKIEKELEKDKSNEKESTEENKEKEETK

>21:06:03|GENSCAN predicted CDS 5|1248_bp

atggcaagcgaagtggcccaaatacccgccgaggaaacgcccgcagtggcggcggaaaaatcagaggagccggaaaa gtcagcggccccgccagcggactcagcggccgctccagctgcgcccccgcagtggagaaggctgaggatgccgatggcga gaagaaggacggcgaggccggaaagcaggacaagcaggatggcgaggagcccaaaaaaggacgaggtggcagc gtaccagttctcgcgcaccccactgctgccctcctgtcgccctactgcctgaaggtggagacctggctgctgtgtgggcctga cgattcggccatcatcatcaaggaactgtcgtccaaatacgagaagtacctggactcgggactcaccgccgagcaaaggaatgt 10 ctcgtacgccacgattgccatgctggagaaccatctcatctggatcatcttctactggcgcgccaagtatccggacaatgtgctcaagggctacaaggtcaacttgcagcacgccctcggcctgcgcccaactcgattctgaacttcttttaagatcacctttggtcgc a aggg cac gaag aag ctgaag gcg cac gg cat cgg igt ccac agc gcg aggag at cgagg ag ttc gg caag gac gac tgg and the same state of the sameaaggtectcagcgagatgctcgactgcaagcctttcttcttcggcgacgagcccaccaccctggatgtggtggccttcgctgtcct 15 ctcgcagctccactatctgtccaaggacattgcgtatccgctgcgcgactacatgaccgagaagtgccccaacttgattggccacgtatctcgcatgaaggacaagtgcttccccgactgggacgagatctgcacgaagctggacctcaatgcgcacattcccaagccag agcccgagaccaaggagggcaaggagggggagaagaaatcaaacgaacaggagggcactgagggcgacaagat

20. Drosophila Gene Hit rescue sequence and TBLASTN with QRF2: failed axon

connections (U21685)

Human Homologue Drosophila EST BLASTX with fax: Metaxin 1 and 2 (Q13505 and AF053551) several including LD31362 (AA951078 similar by BLASTN to

U21685 failed axon connections)

25

Annotated *Drosophila* genome genomic segment AE003527

Annotated *Drosophila* genome Complete gene candidate CG4609 – fax failed axon connectionsconnections

30 Human homologue of Complete gene candidate 4505281

ref]NP_002446.1|pMTX|
metaxin>gi|3024205|sp|Q135

05|MTXN_HUMAN METAXIN (4e-06)

35

Putative function Drosophila fax is a dominant genetic enhancer of the Abl mutant, developmentally expressed in axons of the CNS

40 Confirmation by RNAi Weak reduction of G1 and G2/M peaks indicating fewer cycling cells

104

Line ID 262/20

Category Mitotic defects in brain: metaphase arrest.

(overcondensation, polyploidy, aneuploidy, few anaphases, high

mitotic index, metaphase with bent bipolar spindle)

5 Reversion NR

Map Position 72F

Rescue ID G6E

Rescue Sequence

15 AGATTTTATAAAAACTTATATGAGTAAAAATTTTAAAATTGTGGAGTCAACCT AAATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTT TTTAAATAATATTCCATTATGCTTACGCTCAATTTATGAACAAATGTITTCTCG ATCCTTAGGTAAAGTTTCGAGTTICGCGACTAGATTTATTAAAATTAAGAACA TCTCCATTTATGTTCCC

20

Drosophila EST several including LD28084 (AA949260)

All other results as for line 1466/4

105

Line ID 262/22

Category Mitotic defects in brain: metaphase arrest.

(overcondensation, polyploidy, few anaphases, high mitotic index,

metaphase with bent bipolar spindle)

5 Reversion NR Map Position 72F

Rescue ID F1E

Rescue Sequence 1

10 AGCTGCACGATAGGATATCTTGGCTGGATGCGATTCGCTTTCGGATTCGGATG
GATTCAGGAGCCGCTGTCTCGACACCGCCGCAACCGCTCTCGGGAGTTTGAAA
ATTTGAAATGAGCGGATTCGCGTTGCGAAGGCNAGCTAGCGTTGCAGGCAGT
GTGGCCAGATGCCGCGTGCGAACGTATTCTCGAATGCAATCGGCCGAGTGCA
GATGCACTAAAAAATAACCCACTTCCAGTGACTGGAAATTAAGATCAAGGAAT
15 AGATTTTATAAAAAACTTATATGAGTAAAAATTTTAAAAATTGTGGAGTCAACCT

AAATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTT
TTTAAATAATTCCATTATGCTTACGCTCAATTTATGAACAAATGTTTTCTCG
ATCCTTAGGTAAAGTTTCGAGTTTCGCGACTAGATTTATTAAAATTAAGAACA
TCTCCATTTATG

20

Rescue ID F1P

Rescue Sequence 2

30 CGGGCGCTCCAGCTGCCGCCCCGCAGTGGAGAAGGCTGAGGATGCCGATGG CGAA

Drosophila EST several including LD28084 (AA949260), LD38479 (AI518768)

35 Other results as for line 1466/4

106

Line ID

262/3

Category

Mitotic defects in brain: Metaphase arrest

(overcondensation, polyploidy, aneuploidy, no anaphases, high

mitotic index, metaphase with bipolar spindle)

5 Reversion

NR 72F

Map Position 7

Rescue ID

H3E

Rescue Sequence

15 GATTTTATAAAAACTTATATGAGTAAAAATTTTAAAATTGTGGAGTCAACCTA AATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTTT TTAAATAATATTCCATTATGCTTACGCTCAATTTATGAACAAATGTTTTCTCGA TCCTTAGGTTAAGTTTCGAGTTTCGCGACTAGATTTATTAAAATTAAGAACATC TCCCTTTATGTTC

20

Other results as for line 1466/4

107

Example 24 (Category 3)

Line ID 238/20

Category Mitotic defects in brain: metaphase arrest

(overcondensation, metaphase with bipolar spindle

Reversion NR Map Position 75E1-3

Rescue ID D7E

10 Rescue Sequence

5

TTCAGTCGCGCATTTCACCGTTTCCGAATCGGACGAACCGGGCGTGATTGCTC
TCCTGCTGCTTTCGAGATCGGAGTCCCGATAAGGATATAACTACAACCTAAAG
AGGAATCCAAGCCTCCTCCTGCCGCTAGTTTCGAAAAGTAAATAGAGTACTTG
TTATCAACTGGGGAAGCGGAGATACATAGCTCCGATATTCCTGTGAAAGCCAG

20 TGGGAGTTAGGGAGGCTCTTTACAATGACAACATTGCACCAAAGATGGACTCT CTCTCTAAAATGCATTTCATACCAATATTTACTTT

Drosophila EST several including LP04802 (AI260815)

25 Annotated Drosophila genome genomic segment AE003519

Annotated Drosophila genome Complete gene candidate CG3979 - novel gene with

homology to sodiumdependent dicarboxylate

transporters

30 Human homologue of Complete gene candidate 3e-87 4506979

ref|NP_003975.1|pSLC13A2|

UNKNOWN

>gi|2499523|sp|Q13183|NDC1

_HUMAN RENAL SODIUM/DICARBOXY

40 Putative function sodium/dicarboxylate transporter

Confirmation by RNAi Only WT profiles observed

PCT/GB01/01297 WO 01/72774

108

Line ID

490/9

Category

Meiotic defects in testis: segregation defects, multipolar spindles

(Mul-02/29)

Reversion

NR

Map Position

95C1-8

Rescue ID

I4E

Rescue Sequence

GCTCTGCCGCTTCAACCGCCCGCGTTCTGTGTGTGTGCCGCGACGTAGG TGTAGGGTCCGCTGCACACGTGTGTGTGGGAGCGCGCGAGAGCGGGAGAAGA 10 GCAGAACGTTTTTGGGCGGCTAGTGGTGGCACCGTGAGCATGCCGGTCGTCGT AAGATAGGCTTAGGAACACTCAGAGAAAATTTGTTTAGCTCAGCATTTTCCTA AGATCGGCGATTTAAGCTACGCTTAAAGGGTAATTTGTCTGAGATATCTTTGT CATTTAAAGTTAAGTCTCAGCTTATCCAAAAGTCAGTTATTGGAAAAAAGGAG CCAGCTTTTCAGCAGAGTTCGGCTTAAGCGCTTATTATCATATTAACCAGCTTA ATTAATGTATCTTTTAAATTGTTATATGCATTAAATCACTAATTAAGGTGATTA CCATTTGTACGTTTTAAATTAAAGTATTTTGATTTTCACTAATACAGGCTCTAA GCTGATCCAAATCTACAAGCTTAGTTTTTGAATAGTCTTCACATGTTGACTTTT

ATTCTCT 20

Genomic hit, Accession No. CSC:AC015160

Other results same as 238/20

109

Line ID 660/3

Category Meiotic defects in testis: cytokinesis defects, abnormal spindles.

(Ab-01/03)

Reversion R?

Map Position 75E

Rescue ID H8E

Rescue Sequence

5

20 Genomic hit, Accession No. CSC:AC015160

Other results same as 238/20

110

Example 25 (Category 3)

Line ID

5

15

273/18

Category

Mitotic defects in brain: metaphase arrest

(overcondensation, very high mitotic index, few polyploids,

metaphase with bipolar spindle)

Reversion

NR

Map Position

75E

10 Rescue ID

DIE

Rescue Sequence

CCCATTACCACGCTAATCAACGCGAAATGGACGCAGACGCCCCTATATCTGGA AATCGCCGAGTATCTGGCCGATGAGCAGGCGGGCCTCTTCTGGGATTACGTTT CGGGGGTGACAAAGTTGGACACGGTTCTCAACGAATATGGTTTGTGTTTATAA GTCATGGAGAACCCGCATTAAAGAGCTTTTTATATTCTCCTCAATGTGAATCC

20 GAATCCATATAAAATC

Genomic hit, Accession No. AC015160

Associated ORF

Genscan: >ORF2 predicted sequences

25 >16:57:34|GENSCAN_predicted_peptide_5|1548_aa
MLRAVALCVSVVLIALYTPTSGESSQSYPITTLINAKWTQTPLYLEIAEYLADEQA
GLFWDYVSGVTKLDTVLNEYDTESQQYNAALELVKSHVSSPQLPLLRLVVSMHS
LTPRIQTHFQLAEELRSSGSCQSFTFAQVGSELACSFNELQKKLEVPLAKDSLDAS
VVTYSFDHIFPGSENNTRTVVLYGDLGSSQFRTYHKLLEKEANAGRIRYILRHQLA

- 30 KKDKRPVRLSGYGVELHLKSTEYKSQDDAPKPEAGSTSDEDLANESDVQGFDFK VLKQKHPTLKRALDQLRQRLLQGNDEIAQLKAWEFQDLGLQAAAAIAEIQGDET LQILQYTAHNFPMLARTLLAHKVTDGLRAEVKHNTEAFGRSLNVAPPDGALFING LFFDADTMDLYSLIETLRSEMRVLESLHSNNVRGSLASSLLALDLTASSKKEFAIDI RDTAVOWVNDIENDVQYRRWPSSVMDLLRPTFPGMLRNIRKNVFNLVLVVDAL
- OPTARSVIKLSESFVIHQAPIRLGLVFDARDANEDNLADYVAITCAYNYVSQKKD ARAALSFLTDIYAAVGETKVVTKKDIVKQLTKEFTSLSFAKAEEFLEEDSTYDYGR ELAAEFIQRLGFGDKGQPQALLNGVPMPSNVVTADSDFEEAIFTEIMTHTSNLQKA VYKGELTDNDVAIDYLMNQPHVMPRLNQRILSQEDVKYLDINGVAYKNLGNVG VLNRLSNRDMTATLMDNLKYFGGKKSTELIGRASLQFLTIWVFADLETDQGRDLL
- THALDYVQSGESVRVAFIPNTESSSASSRRNLNRLVWAAMQSLPPTQATEQVLK WLKKPKEKIEIPTQLEDILGSTELHLKMLRVYSQRVLGLNKSQRLVIGNGRLYGPL SSDESFDSADFALLARFSSLQYSDKVRQVLKESAQDVNEEFNSDTLLKLYASLLPR QTKTRFKLPTDLKTDHSVVKLPPKQENLPHFDVAAVLDPASRAAQKLTPILILLRQ VLNCQLNLYLIPVPQHSDMPVKNFYRYVVEPEVQFEANGGRSDGPLAKFSGLPAN
- 45 PLLTQQLQVPENWLVEAVRAVYDLDNIKLTDIGGPVHSEFDLEYLLLEGHCFDAA SGAPPRGLQLVLGTQSQPTLVDTIVMANLGYFQLKANPGAWSLRLREGKSADIYA

WO 01/72774

ISHIEGTNTHHSAGSSEVQVLITSLRSHVVKLRVSKKPGMQQAELLSDDNEQAAQS GMWNSIASSFGGGSANQAATDEDTETINIFSVASGHLYERLLRIMMVSLLKHTKSP VKFWFLKNYLSPQFTDFLPHMASEYNFQYELVQYKWPRWLHQQTEKQRTIWGY KILFLDVLFPLNVRKIIFVDADAIVRTDIKELYDMDLGGAPYAYTPFCDSRKEMEG FRFWKQGYWRSHLMGRRYHISALYVVDLKRFRKIAAGDRLRGQYQALSQDPNS LSNLDQDLPNNMIHQVAIKSLPDDWLWCQTWCSDSNFKTAKVIDLCNNPQTKEA KLTAAQRIVPEWKDYDAELKTLMSRIEDHENSHSRDSAVDDSVDDSVEVTTVTPS HEPKHGEL

>16:57:34|GENSCAN predicted CDS_5|4647_bpatgttacgtgccgtggctttgtgtgtgtctgtggtgctca 10 tage a ctatata cgc caact to tgg ggaat c cag tcag age tate ce at caccae gctaat caac gc gaa at ggac gc ag ac gc caccae act to tgg ggaat cag gaa gc caccae act to tgg ggaat caccae act to tgg gaa act gaa gc caccae act to tgg ggaat caccae act gc caccae act gcggttctcaacgaatatgataccgagtcgcaacagtacaatgccgccttggagctggtcaagagccatgtgagttctccccaattg cccctgcttaggctggtggtatccatgcatagcttgacgccccggatccagacccacttccagttggccgaggaactgaggagca gtggctcttgtcagagctttacttttgcccaggtgggttccgaactggcctgcagctttaacgagctgcagaagaagctggaagtgc 15 cgctcgccaaggatagcttggatgcttctgttgtcacctacagctttgatcacattttccctggcagtgagaacaatacccgcactgtggtactatacggcgatttgggaagctctcaattccgcacctatcacaaactattggaaaaggaagccaatgctggccggattcgtta gctttgatttcaaggtgctgaagcagaagcatcctacacttaagagagcgctggatcaactgcgtcagaggcttcttcagggaaac 20 gatgagatcgcccaattgaaagcatgggagttccaggatttgggtctccaggcggccgctgctattgcagaaatacagggtgatg aaaccctacaaattcttcaatatactgcccataatttccccatgttggccagaaccctgctggcccacaaggttacggatggcttaaggatggctaaggatggcttaaggatggctaaggaggcggaggtaaagcataatacggaagcatttggaagaagcttgaatgtagcgcctccagatggtgcccttttcatcaatggactcttcttcg at gct gacaca at gg at ct gt at tccct gat tg agac gct gcg ctcg gag at gcgt gt tctcg agag tct gcaca gt aat aat aat gat gcg gag at gcg gagtgaggggaagccttgccagctccttgctcgccttggatctgacggcctccagcaaaaaagaattcgccatcgacatccgtgaca25 ttcctgg catgttaag gaatatccgaaa gaatgtgttcaatttggtcctagtggtagacgcgctgcagcccacagctagaagtgttattaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtgtaaggaatgtaaggaatgtgtaaggaatgaatgtaaactgtcagagtcgtttgtcatccatcaagctcccattcgcttgggtttggttttcgatgcgagggacgccaacgaggataatcttg30 cggagacaagggacaacctcaggccttgttgaatggtgttccaatgcccagcaacgttgtgaccgccgatagcgacttcgagga 35 a a get ctt ce ge ctea a geeggagga at ctt a a tegat t g get t ge cat ge a get ctt ce a cea a ctea a gee a get ctt cea cea a ctea a get ctt cea a get ctt cea cea a ctea a get ctt cea a ctea a get ctt cea cea a ctea a get ctt cea cea a ctea a get ctt cea ctea a ctea aggtcctgaaggaatctgctcaagatgtcaatgaggaattcaacagcgatacattgcttaagttgtatgccagcctgcttcccaggcaagtcaagatgtcaatgaggaattcaacagcgatacattgcttaagttgtatgccagcctgcttcccaggcaagatacattgcttaagttgtatgccagcctgcttcccaggcaagatacattgcttaagttgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgcttaagatacattgcttaagattgtatgccagcctgcttcccaggcaagatacattgctaagatacattacattgctaagatacattacattgctaagatacattacattacattgctaagatacattacaaaccaaaactcgctttaagctaccaacggacttaaaaaaccgatcactcggttgtaaaactaccgcccaaacaggagaatcttcccc attttgatgttgccgccgtttttggatcccgcctcccgagcagctcaaaaactaacgccaatacttattttgcttcgtcaagtgctgaactgcca att gaact tatacct gattcccgtcccccagcacagcgatat gcccgt gaagaact tctacagatacgt tgt ggaaccggagaccggagacct gattccccc gattcccccagcacagcgatat gcccgt gaagaact tctacagatacgt tgt ggaaccggagagacct gattcccccagcacagcgatat gcccgt gaagaact tctacagatacgt tgt gaaccggagaact gcccgt gaagaact gcccgt gcccgt gaagaact gcccgt gcccg45 gtcca attcgaggcgaatggaggccgatctgatggtcctttggcca aattcagtggattgccagccaatcctctgctgacccagcaatccaatccagcaatccaatccagcaatccaatccagcaatcctgtgcacagcgaattcgatctggaggttatctgctgttggagggtcactgctttgatgctgctagcggcgctccgcccagaggacttc

agtiggtgttgggtacccagagtcaacctaccttggtagatactattgtgatggcgaatttgggttatttccaactlaaagccaatcca ggagcttggtccctacgcttgcgtgaaggcaaatcggcggatatttatgcaatcagccacattgaaggaacaaatacccatcattc ggctggctcttctgaagttcaggttcttataacctccttgcgatcccatgttgtcaaattaagggtgtctaagaagccaggcatgcag caggcggaactcctgtcagatgacaacgaacaggcagcgcaatcaggcatgtggaacagcatcgccagcagttttggcggcggcagtgccaaccaagcagccactgatgaggatacggaaaccatcaacattttctctgtggcatcgggacacttgtacgaacgtcttcta aggat cat gat ggtt t cgct gctaa ag cacacaaa at cacct gt gaag tt ct ggtt ct t gaag aact at ctt t cgcc gcaat tt ac ggtaag to the state of the stataaaaacagaggaccatttggggctacaagatccttttcctggacgtgctcttcccgctgaatgtgaggaaaatcattttcgtggatgc cgatgccatcgtaagaacggatataaaggagttgtatgacatggacctcggaggagcaccctatgcctacacgccattctgcgatt cccg caa agagatgg agggcttccg attctgg aag caggggatactggcg aag ccatctgatggg caggcgtt accacatttccgccttgtacgtggtggacttgaagagattccgcaagattgcggcaggagataggctaagaggccaataccaggcacttagccaggatccgaacagcttatccaatttggatcaggacttgcccaacaacatgatccaccaggtcgccatcaaatccctgcccgacgactgg ccaaactcacggccgcccagaggattgtgcccgaatggaaggactacgatgccgagctgaagaccctgatgtctcgcatcgag gat cat gaga at teget age category and the desired control of thegagcccaagcacggcgagctgtga

Drosophila Gene Hit rescue sequence and BLASTX with EST and TBLASTN with ORF2: UDP-glucose:glycoprotein glucosyltransferase (U20554)

20 Human Homologue BLASTX with UE Drosophila EST several including G

5

10

15

30

35

BLASTX with UDP-GGT: hypothetical protein (AL133051)

several including GH16576 (AI293351)

Annotated *Drosophila* genome genomic segment AE003519

Annotated *Drosophila* genome Complete gene candidate ugtUDP-glucose-glycoprotein glucosyltransferase

Human homologue of Complete gene candidate CG6850-

IGI_M1_ctg14521_41 D65BCE6EEC187AE3

TRANS:SEPT20T.ctg14521.2

2 FPC_ctg:ctg14521 FPC_start:1284609 FPC_end:1284696

FPC strand:+ (1.20E-215)

Putative function ugtUDP-glucose-glycoprotein glucosyltransferase

40 Confirmation by RNAi Only wild type profiles observed

113

Example 26 (Category 3)

Line ID

430/5

Category

Mitotic defects in brain: metaphase arrest

5

(overcondensation, polyploidy, metaphase with bipolar spindle)

Reversion

NR 98B5-8

Map Position

Rescue ID

2C2E

10 Rescue Sequence

20 GGCTTGGCCAGCACCTTGGCCACGGTGCGCTTCTCGGCGGCGAGGGCGGCAC GACGCTTGAGTACCTCGGCATAAGGGTTCAACTTGATCAACTTGCGCACGGTT GGTAAGGGGGTT

Drosophila EST

several including LD45359 (AI513164)

25

15

Annotated *Drosophila* genome genomic segment AE003763

Annotated *Drosophila* genome Complete gene candidate CG5502 RpL1 - Ribosomal protein L1

30 Human homologue of Complete gene candidate

1e-126 432359

dbj|BAA04887| (D23660) ribosomal protein [Homo

sapiens]

35

Putative function

structural protein of ribosome involved in protein

biosynthesis

Confirmation by RNAi

Marked decrease in G1 and G2/M indicating fewer cycling

cells

114

Example 27 (Category 3)

Line ID

5

472/12

Category

Mitotic defects in brain: metaphase arrest. Meiotic defects in testis:

segregation defects. Abnormal spindles

(mitotic: High mitotic index, meiotic: Ab-08/24) R?

Reversion
Map Position

96C7-9

Rescue ID

2B6E

10 Rescue Sequence 1

GTCTGACGTTCTCTGAGGGCAAAAGTTTCGAGTTAGTTGAAGGTGAGGGTGCT
CGATCACCGATTTGCGGTGAGACGAAAGAAAAGTATGCATTGTTGCGTTGTAA
AGAGAGCCGGCGCTCGTCTTGTTCACATTGTCGCTGAGAACGTATGTTGTGCT
TCATCATTTCCTTGTTGATTTCCCTTTGACGTGGCAACTTGACCATGTATGACA
ACTCTTTGGTGGTGCCATCTGGAAGGCAGAAATTTGATGTCAACGGTGCTCCC
AGCCAGTCCACTCCCCAACTCACCCTGCAGCTCCACTTCGATATTAACTCGCA
ACATATTAGTGGCGTAGTTGTCACCTGCCGCGGATCCCATTTCCGCTTTGAAAT
TTCGCACTTTCGAATATCCGTCCACATTCGATTTGAGAACATCTTCGAAACGTT
CAGCGGTGACCCAATCGGGTATTTTGCCAGCCGCCATTGTAGATAATCGGGAT
AAGTATTTTGAAATCGAGCAGAAAACACATATACGTCCAGTGTGACGTCTTG
CGTAGACTGATGAAAGCCGAGTATTAGACTCTACACATCTGTGGAGCTTTTTA
ATTTCGTAGTGCGCGGCCGATTTCTCTCCGATCTTCTCAAAAGCTCCGCTAAT

Annotated *Drosophila* genome genomic segment AE003751

25 Annotated *Drosophila* genome Complete gene candidate CG10618 - novel Human homologue of Complete gene candidate none

Putative function

no homologies which indicate function

30

Confirmation by RNAi

Only wild type profiles observed

PCT/GB01/01297

115

Example 28 (Category 3)

Line ID

WO 01/72774

571/15

Category

Mitotic defects in brain: metaphase arrest

5

15

30

(overcondensation, few anaphases, some polyploids)

Reversion

NR 93D

Map Position

Rescue ID

2A8E

10 Rescue Sequence

GGCGGCGCTACATTTGTTGTTGTCGCTGCTGCTCACAGCTCCACCACCATTTGC ACAGTTATATTACCTCGCTCAAGTCGCCCCTCTCCCTCTCGCCCACTCGCTGTG TCAATCGAATTAAAACGAATGCTCTTCGGCGAATAATTGGGTTTAGATACTTT TCCAGCAGACAAAGTTGTATTTTTTGCACTTCTTATTGATATTAGGCAAAACGC ATCGGCCGAATCACACGCACACAAAGCACACACGCGAGCAGCGGTTTTTCAA TCTGCAGTACACCAAACAACACACACTATTTCCTAATGCCTGTTCTTATCCCTC TGATATTCCCAATGAATCGCTGGGCAATTGGCGATTCGAACCGATTTTCACTT GGCTCTTTGTTTTATTTAATTTTCACCGAAACGCTCTCACACGCAGAGACGCTT TTGCTCGTTCGCTGATGCTTCTGCTGCAATACACACCACCTACGAAACGAGCC

AAGGGAAATTGTATCTATGGGCTGTGTATCTGTTTCTACGCGGCACGCGCTGC 20 ACGTCCGCTCGCTTCGGGTTTTCGAGAGAGAATATAACTTTTTCGATACGGTA CGGTAAACGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCGTGATA CGCCTATTTTATAGGGTAATGCATGATAATAATGGGTTCTTAGACGTCA

25 Drosophila EST

LP07504 (AI294185), LP06548 (AI293427)

Annotated Drosophila genome genomic segment

AE003734

none

Annotated Drosophila genome Complete gene candidate CG15802 - novel homology

to Doom, a product of the Drosophila mod(mdg4) gene, induces apoptosis and binds to

baculovirus inhibitor-of-

apoptosis proteins

35 Human homologue of Complete gene candidate

Putative function

inducer of apoptosis

Confirmation by RNAi

Only wild type profiles observed

116

Example 29 (Category 3)

Line ID 736/15

Category Mitotic defects in brain: prometaphase arrest

(overcondensation, fewer anaphases, metaphase with bipolar

5 spindle)

Reversion NR Map Position 73C

Rescue ID H5E

10 Rescue Sequence

30

35

20 Genomic hit, Accession No. CSC:AC014181

Annotated Drosophila genome genomic segment AE003526

Annotated Drosophila genome Complete gene candidate CG3971 baldspot - with

homology to membrane

25 glycoprotein

Human homologue of Complete gene candidate CG3791-9e-08

4680391emb|CAB41293.1| (AL034374) dJ483K16.1 (novel protein) [Homo

sapiens]

Putative function membrane protein, function unknown

Confirmation by RNAi Slight reduction of G1 and G2/M peaks indicating fewer

cycling cells

117

Example 30 (Category 3)

Line 1D

Category

5

Mitotic defects in brain: metaphase arrest

(condensation, no polyploidy, no anaphases, metaphase with bipolar spindle)

Reversion

NR

Map Position

100D

Rescue ID 10

2E3E

Rescue Sequence

GGTCAAGCCCGATGGCGTCCAGCGCGGGCTCGTCGGCAAGATCATCGAGCGC TTCGAGCAGAAGGGCTTCAAGCTGGTCGCCCTGAAGTTCACCTGGGTAAGCGG ATAATTGAATTAGGAAGAAATCAATAGATATACATACGTGGAAACGGGTTGC CCCACGCGGGGTTGCTATCGGACCTAACCTCAAAGGCTGGGTGCAGGCGTCAT

- CGCGGAATGACATGTTTTAGAGGTCAGAACTGCAATTAACTGATAACGAACC GTTTTGTAACCAGGCCTCCAAGGAGCTGCTGGAGAAGCACTACGCTGATCTGT CCGCCCGCCCTTCTTCCCCGGACTCGTGAACTACATGAACTCCGGCCCCGTG GTGCCCATGGTGTGGGAGGGTCTGAATGTGGTCAAGACCGGTCGCCAGATGCT
- CGGCGCCACCAACCCCGCCGACTCGCTGCCCGGCACCATCCGCGGTGACTTCT 20 GCATTCAGGTCGGACGCAACATCATCCACGGCTCCGATGCCGTCGAGTCTGCC ĠAGAAGGAGATCGCCTGTGGTTCAACGAAAAGGAGCTGGTCACCTGGACCCC GG
- Genomic hit, Accession No. CSC:AC012727 25

Associated ORF

Genscan ORF1 predicted sequences >16:43:49|GENSCAN_predicted_peptide_7|172_aa MKLLMLGTILAFFSVISATMAANKERTFIMVKPDGVORGLVGKIIERFEOKGFKLV ALKFTWASKELLEKHYADLSARPFFPGLVNYMNSGPVVPMVWEGLNVVKTGRQ 30 MLGATNPADSLPGTIRGDFCIQVGRNIIHGSDAVESAEKEIALWFNEKELVTWTPA **AKDWIYE**

>16:43:49|GENSCAN predicted_CDS_7|519_bp

35 ggtcaagcccgatggcgtccagcggggctcgtcggcaagatcatcgagcgcttcgagcagaagggcttcaagctggtcgccc ctacatgaactccggccccgtggtgcccatggtgtgggagggtctgaatgtggtcaagaccggtcgccagatgctcggcgccac caaccccgccgactcgctgcccggcaccatccgcggtgacttctgcattcaggtcggacgcaacatcatccacggctccgatgccgtcgagtctgccgagaaggagatcgccctgtggttcaacgaaaaggagctggtcacctggaccccggccgaaggactgg 40 atctacgaatag

Drosophila Gene Hit rescue sequence and TBLA: abnormal wing disc (awd) (X13107) Human Homologue BLASTX with awd and TBLASTN with ORF1: tumor metastasis inhibitor nm23-H2 (A49798) non-metastatic cells 2, protein (NM23B) (P22392) and nucleoside diphosphate kinase B.

118

Drosophila EST several including LP05977 (AI257573 similar by TBLASTX to X92956 B.taurus mRNA for nucleoside diphosphate kinase (NBRA)

Annotated Drosophila genome genomic segment AE003779

5 Annotated *Drosophila* genome genomic segment AE003779
Annotated *Drosophila* genome Complete gene candidate CG2210 - awd abnormal wing discs nucleoside diphosphate kinase

10 Human homologue of Complete gene candidate gi4505409

1A5C3F84D7AD272C |ref|NP_002503.1| nonmetastatic cells 2, protein (NM23B) expressed in [Homo saniens] (190F-61)

15 sapiens] (1.90E-61)

Putative function human nucleoside diphosphate kinase, transcriptional regulation of c-myc expression.a candidate suppressor of tumor metastasis

Confirmation by RNAi Only wild type profiles observed

CATEGORY 4: ANAPHASE DEFECT

Example 31 (Category 4)

Line ID

1132/8

5 Category Mitotic defects in brain: anaphase defects

(overcondensation, high polyploidy, some lagging chromosomes)

Reversion

Map Position

86F3-6

10 Rescue ID 2C3E

Rescue Sequence

GGCCGGAGGTACCATTTTGGTAGGACCGTTTTTCGGGCCAACGAAAATACCAC AAGACGGCAGCGATAATAGTGTTTTTTGCTTCAAATGTAGTATGGCTACGCAA CTCACATATGGTTAAGAACTTCGCTGTTTATTTGGTGGTTAAACTAGCTAAATA

- CAATAAGAGTGGCAACGCCGTCACGTTTTCTACATGTATTTTACTTGGCGTAGT 15 GCGCCAAGCTTATAAACCACAGTTGGGCGGTTCTTŢTGAATTGTTTAATTTACA AGAATACGTTTACTCAAGGTTCGCAGCTTGTCAATCAGTATTCGCAAATATCA ATAATAAAAGGCATCAATTTTCCAATCAGCAGTTGAAAAGAACTCCCCTCGAC
- ATTTGAACAAAATGCATTTTTGGGTGATTATAATTTATTAGAATTTTTATTGAC TAATANTATTTGGATTCAAGGAAAATATATTTCAAAATGGCGGGGTTTAATA AAACAATTTTTCAAATTAAGG
- Genomic hit, Accession No. AC007805 25

Drosophila EST

several ESTs including LP09688 (Al295922)

Annotated Drosophila genome genomic segment

AE003693

Annotated Drosophila genome Complete gene candidate CG6929 - Lk6 kinase 30

Human homologue of Complete gene candidate

gi4505191

DB39E49EC0BED990 |ref|NP 003675.1| MAP

35

kinase interacting kinase 1 [Homo sapiens] (6.20E-113)

and gi9994197

551A82FA3D09FD58

|ref|NP_060042.1| G proteincoupled receptor kinase 7

40

[Homo sapiens] (1.70E-106)

Putative function

Protein kinase associated with microtubules

120

Confirmation by RNAi Cocells

Complete loss of G1 and G2/M indicating fewer cycling

PCT/GB01/01297 WO 01/72774

121

Line 1D 483/19

Meiotic defects in testis: segregation defects Category

Reversion 86F Map Position

5 H2S Rescue ID

Rescue Sequence 1

CTCCGGCCACACGGATGAATTCGTCGTCATTCGTCGGAATCATTCGAACTTTG AAAATGGATCGGTAGCTGGGAAGGAAACTTAAAGCGAAATACGCAAAGAAA ACGGCTTTTGTCCGCTATTCAGCGATTTTTTTTTGTGTTGTAATCAGCAGAGGAA 10 ATTTTAACGACCAACTCCACCGCCACACCAGCCATCTCCAGCAGCCCCGGAAA ATAAAATAGAACTAAATTAACGCCACCATCACTACAACAACCATCTCACCAAC AACTACAAGAGCAACAACCACAGCAACAGCACTACTGCACCAAGCCCACAAA ATATCGCAGATAACCGAAAAAAGCGGTGCAATAGATAAACCCCATTTTTTGCT

TGAGCTTTTTCGCCTGTGTGATGAGAGAAATCAGCAGCAGCCATCGATTACA ACAACAACAGCAGCCACCAACGACGACTCACCACCAAACGAAGAATAATA ACCAGCGGANAGCGATAGATA

Genomic hit, Accession No. 20

CSC:AC018284

Drosophila EST

several including GH28825 (AI517767), LP04213

Other results same as 1132/8

122

Example 32 (Category 4)

Line ID

1422/14

5 Category

Male and female sterile, small wings, meiotic defects in testis:

segregation defects, elongation defect

Reversion

NR

Map Position

90B4-8

10 Rescue ID

2F1E

Rescue Sequence

GGCCAGCTGCTCAAACATTCTGCAGCTATTTGGCCGCCAGCGAGTAGAACGAT ATTGCCAAATATTTTATAATAGTAACCAATACGTTACCAGTATGACCGCGCCG ATAACGATAGAAAATACCACACGGTCTAAAAGTAAATACCATTTGGGGTATTC

- 20 ATAAAACTGGTAATTAACAAAAGTAAAAAGTTACTTAACTTATACAAAAATAT TTAGTTATTGNATTCAATAATAAGATGGTAATAATAGATGGTAAGATAGTAAT ATTTTAATAATTGAATTTCATCACACATGCTGGTGCACGTTCCACAACTTACAA TCAAACGAAA
- Annotated Drosophila genome genomic segment AE003718
 Annotated Drosophila genome Complete gene candidate CG7623 novel with homology to UDP-galactose transporter.
- 30 Human homologue of Complete gene candidate 2136348 UDP-galactose transporter related isozyme 3 human >gi|1669564|dbj|BAA13527| (1e-36)

35

Putative function

sugar modification protein

Confirmation by RNAi

Slightly reduced G2/M

PCT/GB01/01297 WO 01/72774

123

Example 33 (Category 4)

Line ID

1479/10

Category

Mitotic defects in brain: anaphase defects

(overcondensation, anaphase bridge, metaphase with swollen

chromosomes and bipolar spindle)

Reversion

5

15

NR

Map Position

69F3-7

Rescue ID 2D6E

Rescue Sequence 1 10

CCACGGCCAAATGTGGTCCGGAGGTCCACGACAACGTGCCGCTGACCATATC CCAGATTGAGCGCGCAACTCAGGATCCGGAGAACGAGAATGTGTTCATCACA GACGACGTGCATCCGATTCACTTCTGCACCTGCATCATCTACGCCTTTGTAACT GGCAATGGAACGCACAACGAGTCGTTCATGAAGTTCATGATCGATGATGGCA CCGGCTCCCTGGAGGCCAGCATCACCAAAAAACCCTTCAATGGACGCGTGATC

AGCAGCCTGTACAGTGAAGCCAGTTCGCTGGCCTCGTCCGAGGCCTACAAGA GCATTGCCGTGAGCATGATGCGGCTGCTGCAGGTCTCCATGGAGTACATTGAT CCCACGCGCATCTCGAGGGGCCACAGCCTATTCCTGCGCGGTCGTCCGAATAG GTTCCGCGGCAAGATGGGTCTGGACGCTTTTCAGTTCTTCATAGACAGCGGCC GATCGCGGAATATGGAAATTGGCTTCGTGGACTACCTAACCGACTGGCAACG

20 AAGGCATAAAACAATGCAAAATAC

Rescue ID 2D6P

Rescue Sequence 2

GCCCGTGGACTTTTCACTCTGTTGATTCTTGCGTATCACGAAGTTATCCAGCTG 25 GCTTTCTATGTCCTCGAAACTCTGATTAAAATCCATTCTATTTGCTTAGTCTGC GATTTCAAAGGGGATTTCTTTATTGCAGTGCATTTTGCATTAGCGCCAAAAAA AAAAAGTTGTGAGCATGGGCGTAGACTTCGTATTTTCTTACAAATAATATTA ATTAAAATTAATTTTGTGAGCAATTTTCACACAATTGTATTATAAGTTAAAACC

AGGGTCACATTAATTTGCAGAACCGCGCAATATTTTCTTTTTAACCCCCTTACA AATTTTCAGTTGTTTTGACTACGCCCCTGCTAATTTTTACTTATTAAATTCAAA GTCTAAAAACATTGTCACCAGATAATACGAGTATACACTATATGGACAAACGT AAAATCGTTAATAGAATATATATATTCAACCATTATTTCACCACCGAGAGAAA TTCATTTGCACAAAACGCCAGGTTGGCAGCACCATCATTGCGCACAGCAAGTG

GGCAAACTCGTTGTATCGCTTG 35

Genomic hit, Accession No. AC007328

Associated ORF

Genscan ORF1 predicted sequences >17:42:01|GENSCAN_predicted_peptide_2|1507_aa 40 MKLAPTVKLNNGYEMPILGLGTYNLKKSRCEAAVCHALEMGYRHIDTAYLYRNE GIIGKVLAKLIGDQKLKREQVFLVTKLWDIYHEPKMVKYACDMQLKLLGVDYID LYLMHSPVGVDYISDEDLMPHENGQLRTNDVDYVDTYRSMEQLVHLGLVRSLG LSNFNANQLKRLLENCQIKPANLQIECHPELVQVPLIELCKFHNITVVAYSPLGRSQ

TCNPLPDYYTDSKLLALAAKYGKTPAQIILRYLSKDNEGEAAVKHAIDVGYRHID 45 TAYFYQNEAEVGKAIRDKIAEGVVKREDIFLVTKLWNIFHDPERVEGICRKQLSNF GLDYIDLYLMHMPVGYKYVDDNTLLPKNEDDVLQLSDVDYLDTYKAMEKLVKL

GLRIEQLAGLSHLSTHSDGMQFRIRMFLTFQRGGPSHNNMQQQQQRGGGSGTDF YNQQRDRRDSGRQMDNNYSNNYNNNNNNQRNRGGGNGMQQQQRGGNGGSGG GGGNGGGNNPAWNMHRGNQNSNNMMNMRNRGMGSRGPMRPNQVHLLVTHT AIDGLLNPGFHILQGYRPQSANNQNKPRNKIKFEGDFDFEQANNKFEELRSQLAKL KVAEDGAPKPATNATAATATATNEQVGEKVEGVHTLNGETDKKDDSGNETGAG EHEPEEDDVAVCYDKTKSFFDNISCEAAQDRSKNKKNDWRQERKLNTETFGVSS TRRGSVAHQLNVFQAVTADATNTTTIMATAALTRDMEERQATTGTIIAWVGGGG NFRNRSNNRNNGGGRGGNGMPNITNGNTAAALKAANNAAGHGSNATDSSAPNA TTATTKSTSLLPEQTQQVAAVSLPVLLPSIGWLFIVMDGPPDIPRSADIAILFVSFEQ SVLFLKFHKRYNEFAHLLCAMMSFEDIESQLDNFVIRKNQQSEKSTGKCGPEVHD 10 NVPLTISQIERATQDPENENVFITDDVHPIHFCTCIIYAFVTGNGTHNESFMKFMID DGTGSLEASITKKPFNGRVISSLYSEASSLASSEAYKSIAVSMMRLLQVSMEYIDPT RISRGHSLFLRGRPNRFRGKMGVCTNATAPSVSSINRILRNRAAERAAAEFARAAS YGYAIHPTHPHPYTSFPTWPAHHPLWGAVPLATPPGGGPAGAGGALQPGGSGSSY GSDGNMSSNPNSSNSNTTHSNGHNTNSGSGCGDSSAGSGRLSLPALSPDSGSRDS 15 RSPDADANRMIDIEGEDSESQDSDQPKFRRNRTTFSPEQLDELEKEFDKSHYPCVN TREKLAARTALSEARVQVWFSNRRAKWRRHQRVNLIKQRDSPSTSSSPTPLVNPV VSPVSPIPVPVAVPESGQQKQPYPYSTSNMCNTSSSSSNSQPCNTINPGSKMSSK TSSVSSNQHMEEPAAAVATASPTASAPLSMGGENSAFRALPMTLPMPMTLPTASA AAFALSFARQYIAKTLLGSPPRSQPPTTNQHKPEPNREFLNEACSSAASVQNSTTP 20 ATTADTPTAKSAMCVHCEKKGGAMEWM

>17:42:01|GENSCAN_predicted_CDS_2|4524_bp

tgtgaggctgccgtgtgccacgccctcgaaatgggctatcggcatatagacaccgcatatctgtacaggaatgaaggcattataggcataggcatataggcataggcatataggcata25 gaacccaagatggtgaaatacgcctgtgatatgcaattaaagctactgggcgtggactatatagatctatatctgatgcattcgccggtgggcgtggactacatctctgatgaagatctgatgcccacgagaatggccagctgaggaccaacgatgtggactatgtgactatgtggactatgtggactatgtggactatgtggactatgtattactggaaaactgccaaatcaagccggcaaacctacaaatagaatgtcatccggaattggtgcaagtcccattaattgagctctg30 aaactactggcgttggcagcgaaatacggcaagacaccagctcaaatcatcctaagatacttgtcgaaggacaacgaaggcgaa gccgctgtgaaacatgcgattgatgtgggctatcgtcatatagatacggcctatttctaccaaaacgaggccgaagtgggcaaggcgattcgggacaagatcgcagaaggtgtggtcaagcgagaggatatatttttggtcactaagctttggaacattttccacgatccagaagcgcgttgagggcatttgccgcaagcagttaagcaattttggcttggactatatcgatctgtatctgatgcatatgccagtgggcta35 caaatatgtagatgacaacaccctgctgcccaaaaatgaggacgatgtgctccaactgagcgatgtcgactatctggatacgtaca aagccatggaaaagctggtaaaactgggcctgcgtatcgaacaacttgctggcctgagtcatctttcaactcattcagatggcatgc agtttcggatacggatgtttctaacattccaacgtggcggacccagccacaacaatatgcagcagcagcagcaacgaggcggcg gcagtggaacggacttctataaccagcagcgggatcgtcggggactccggacgtcaaatggacaacaactatagcaacaactaca acaacaataataataataatcagcgcaatcgcggcggcggcaacggaatgcaacagcagcagcgggggggaaacggcggcagc 40 ggcggcggtggaaacggaggtggaaacaacccggcctggaacatgcatcgcggcaaccagaactcgaacaacatgatgggtttattaaaccctggctttcacattttgcagggctatcgtccgcagtcggccaataatcagaacaagccgcggaacaagatcaa 45 aggatgatgttgctgtgtgctacgacaagaccaaatcgttcttcgacaacatctcgtgcgaggctgcccaggatcgcagcaagaa

atcaactgaatgtattccaagcagttaccgcggacgcaaccaatactacaacaataatggcaacggcggcaltaactcgggatatg aacaaceecegcggtcgtggcggaaacggaatgccaaacatcaccaatggcaacacggctgctgctgcaggcgccaac aatgctgctggccacggatccaatgccacggactccagtgcaccaaatgccacaaccgcgacgacaaagtcgacgtcctcttg cagacattccaagatcggcagatattgcgattctcttcgttagttlfgaacaaagtgtacttttccttaaatttcacaagcgatacaacg agtttgcccacttgctgtgcgcaatgatgagtttcgaggacatagaaagccagctggataacttcgtgatacgcaagaatcaacag agtgaaaagtccacgggcaaatgtggtccggaggtccacgacacgtgccgctgaccatatcccagattgagcgcgcaactca ggatccggagaacgagaatgtgttcatcacagacgacgtgcatccgattcacttctgcacctgcatcatctacgcctttgtaactgg 10 caatggaacgcacaacgagtcgttcatgaagttcatgatcgatgatggcaccggctccctggaggccagcatcaccaaaaaaacc cttcaatggacgcgtgatcagcagcctgtacagtgaagccagttcgctggcctcgtccgaggcctacaagagcattgccgtgagc atgatgcggctgctgcaggtctccatggagtacattgatcccacgcgcatctcgaggggccacagcctattcctgcgcggtcgtc cgaataggttccgcggcaagatgggtgtctgcaccaatgccactgctccttcggtgagcagcatcaatcgcatattgcgtaatcga 15 agtttccccacttggccggcgcatcatccgctgtggggagccgtgcccctggccaccgccacctggtggcggccctgctggagccggtggtgcactgcagccgggcggcagtggcagcagctatggcagtgatggcaacatgagctcaaatcccaatagcagcaaca gcaacaccaccacagcaatggccacaataccaacagcggcagtggatggggggatagtagtgccggaagtggacgcctctc cgaggacagcgagtcgcaggacagtgaccagccgaagttccggcgcaatcgcaccaccttcagtccggagcagctggatgag 20 ctggagaaggagttcgacaagtcgcactatccctgcgtgaatacccgcgagaaactggccgcccggacggcactgagcgagg ccagggtgcaggtttggttttccaacagacgagcgaaatggcggcgcaccagcgggtcaacttgatcaagcagcgggactcg ccctcg a catcg agctcacccaeg ccgttgg tcaatccggtggt cagtccggtcagtccagttccagttccagttgcagttccaga at ctggccaacagaag cagcatatccgtacagcaccagcaacatgtgcaacaccagcagcagcagcagcaacagtcaaccgtgcaacaccatcaatcccggcagcaaaatgagcagcaaaaccagcagcgtcagcagcaaccagcaccatggaagagc 25 cagcagcagcagtggccactgcctcaccaagcatcagctccattatcaatgggcggtgagaacagtgcatttcgcgctctgcc catgacettgccgatgcccatgacettgcccacggcatcggcggcgccttcgcgctcagcttcgccgccagtacatagccaa gacgenteteggttetecagateceageteceagecaecaaceaceageataagecegagecaaategegagtteeteaat gaagcctgcagctccgcagcatctgtccagaattcgacaacgccggcaacaaccgcagatactcctacagccaaatcagcaatg tgcgtgcactgcgagaaaaagggagggccatggagtggatgtga

30

Drosophila Gene Hit BLASTN with rescue sequence 2: Histone acetyltransferase GCN5 (AF029776) very small match at end, TBLASTN with ORF1: middle domain histone acetyltransferase GCN5 (AF029776).

Genomic matches histone acetyltransferase

35

40

Annotated Drosophila genome genomic segment
Annotated Drosophila genome Complete gene candidate CG4107 -Pcaf /GCN5 histone acetyl transferase transcriptional activator protein

Human homologue of Complete gene candidate

Human homologue of Complete gene candidate

gi6382076
72F516F8BD10CD0C
[ref]NP_003875.2] p300/CBP-associated factor [Homo

sapiens] (1.20E-197)

45

Putative function Transcriptional activator

126

Confirmation in RNAi Only wild type profiles observed

127

Example 34 (Category 4)

Line ID

184/5

Category

Mitotic defects in brain: Anaphase defects.

5

10

(overcondensation, aneuploidy, some lagging chromosomes and

breaks]

Reversion

R 71B

Map Position
Rescue ID

C4E

Rescue Sequence

15 ATTTTTATGTAAACAGTATTAGCTTTACATGAGATTACCAAATTGTGAGTGTCT GTGTTTGTCTTTTAAAAACTTTAAAAGCACATAAAGAAATATATTTTAAA TTTAATTAAAAAGTTCGTAAAAAGTAAAAGGTAGCTAAATTAAAAAAGTTTCCT ATTCAAATCAGATTTGGCGAACAAAGAGCCAAGTTGGCAACACTGACAATGA CTCCAAGCGCGAACAAAGCGATTTCTATCGTTATCCCACTCTCTCCCAGAG

20 ATCGTTCTCAAGGCCAAATGGAAGGGACTTCGAGACAATTTCCGTGTGGAGTC AAAAGGATCCGGCGGCCGAATAACGG

Genomic hit, Accession No. CSC:AC019852

25 Associated ORF

Genscan ORF1 predicted sequences >22:43:26|GENSCAN_predicted_peptide_2|1003_aa MAPKKSTIVLNVEQFIHDIEERPAIWNRNFHCNKAFLEQMWDELSGAHKLPKIVL KAKWKGLRDNFRVEYKRIPRADNGDFMVDPATFESKWLHYYALLFLTDHMRHR LPKNEQDQSFYFSQQSEDCEKTVVEPDLTNGLIRRLQDSDEDYDEEEMEADGEAS

- EATMEETMPTPPAAHQMNQVSTTPLATGALRAQEEAHQHALIKAGLLRAQLMEL EKEAEDLSRKPPPPQQMTSPVAPSLQVLVEPPAAHCSPPPMVTTTSAQVQQPGSA AVLAPATTTSASSVSSNGAPMGGKRSVSPPPLYNKAHHPLATLAAAHLAAKDRN EDFGPTSAVGGNGDHLSFTQHSYANGLIPALKLKRPRLSEDSNFNGSSTMDTPLVP EDDDYHYLLSLHPYMKQLTAAQKLRIRTKIQKLIFKELYKEDLEESNLDREVYVL
- 35 DDGAEVDLDLGNYERFLDVTLHRDNNITTGKIYKLVIEKERTGEYLGKTVQVVPH ITDAIQEWVERVAQTPVQGSSKPQVCIVELGGTIGDIEGMPFVEAFRQFQFRVKRE NFCLAHVSLVPLPKATGEPKTKPTQSSVRELRGCGLSPDLIVCRSEKPIGLEVKEKI SNFCHVGPDQVICIHDLNSIYHVPLLMEQNGVIEYLNERLQLNIDMSKRTKCLQQ WRDLARRTETVRREVCIAVVGKYTKFTDSYASVVKALQHAALAVNRKLELVFIE
- 40 SCLLEEETLHSEPSKYHKEWQKLCDSHGILVPGGFGSRGMEGKIRACQWARENQ KPLLGICLGLQAAVIEFARNKLGLKDANTTEIDPNTANALVIDMPEHHTGQLGGT MRLGKRITVFSDGPSVIRQLYGNPKSVQERHRHRYEVNPKYVHLLEEQGMRFVG TDVDKTRMEIIELSGHPYFVATQYHPEYLSRPLKPSPPFLGLILASVDRLNQYIQRG CRLSPRQLSDASSDEEDSVVGLAGATKSLSSLKIPITPTNGISKSCNGSISTSDSEGA
- 45 CGGVDPTNGHK

>22:43:26|GENSCAN predicted CDS 2|3012_bp

atggcgccanaaaagtccaccattgtgctcaatgtggagcagtttattcacgacatcgaggagcgcccggccatctggaaccgca atttccactgcaacaaggccttcctcgagcagatgtgggacgagctgagcggagcgcacaaactgccaaagatcgtgctcaagg 5 gatgcccacgccaccggctgcgcatcaaatgaatcaagttagcaccacaccactggccaccggagctttgcgagcccaagaag gaaagccacctccgccacagcaaatgacatctccagtggcaccctcactacaagtgctagtggaaccaccagccgcacactgtt 10 ctccaccgccaatggtgaccaccacatccgcacaagtacaacaaccgggctcagcagctgttctggcgccggcaacgaccacatccgcgtcatctgtatcctcgaatggagcgccaatgggcggcaagagatctgtgtcgccaccgcctctatacaacaaagcacaccatccgctggccactctggcagcagcacatcttgcggccaaagaccgaaatgaggatttcggacccacctctgctgtaggaggaa ggatagcaattttaatggttcctcgacaatggacactccgctcgtaccagaggacgatgactaccactacttgctcagcctacatcc 15 gtacatgaagcagctgaccgcagcccagaagctgcgcatacgcaccaagatacaaaagctcatcttcaaggaactctacaaaga agatettgaggagtccaacctagategegaggtttacgttttggacgatggegeegaggtggatetggatetgggaaactatgaac ggtttttggatgttaccctgcatcgggacaacaacataaccaccggaaaaatttacaagttggtcattgagaaggagcgcactggc gagtacttgggcaaaacggttcaagttgtcccacacatcactgatgccattcaggaatgggtggagcgcgtggcccagacaccc gttcagggatcttcaaagccacaggtgtgcatcgtggaattgggaggaacgattggtgacatcgaaggcatgcctttcgtagagg 20 ccttccgtcagtttcagttccgcgtaaagagagagaacttctgtttggcccatgtgtcgctggttccgttgccaaaggctaccggag aacccaagaccaagcccacacaaagttcggtcagagaactgagaggatgtggcctgagtcccgatttgattgtctgccgatcgga gaaacccattggactggaggtcaaggagaagatcagcaacttttgtcatgtggggccggatcaggtgatatgcatccacgatttga actccatttatcatgttccgctgctgatggagcagaatggtgttattgaatacctaaatgagcgcctacagcttaatatcgacatgagc aagaggaccaaatgcttgcagcaatggcgagatttggcgcgtcgaacggagaccgttcgccgtgaagtttgcatcgccgtcgtg 25 ggaaagtacaccaagttcacggattcgtacgcctccgtagttaaagccctgcaacatgccgccctggcagtgaatcgcaaactgg atgcgatagccatggcatcctagtccccggtggattcggttcccgtggaatggagggcaagattcgtgcatgccaatgggcgcga gagaatcaaaagccattgcttggcatctgcttgggtctgcaagcggcggtcattgaattcgcacgaaataaacttggtctcaaggat gcaaacaccacagaaatcgatccgaacacagctaatgccttggtcatcgatatgccagagcatcacacgggtcaattgggcggc 30 actatgcgcttgggcaagcgaataactgttttctctgatggtcctagtgtcattcgccagttgtatggcaatccgaaaagcgtgcagg agcgt categg categt tae gaggt taat cccaa at acgt g catet g ctg gaa gag caa g g categ gatt t g t g g caceg acgt g categories.cgacaaaactaggatggaaatcattgagctcagcggtcatccctactttgttgccacccaatatcatccagagtacttgtcgcggcc totga agccgtcgcctcctttcctcggcctgatcctggcctcagtggatcgattgaaccaatatattcagcgcggttgccgcctgtcg35 ccccgccagctatccgacgcatcctcggatgaggaggacagtgttgtgggcttggccggagcaacaaaatcgctgagctccttg aaaattcccattacacccacaaatggaatatcaaaaagttgcaatggtagcataagcacttccgacagcgaaggtgcctgcggag gcgttgatcctaccaatggccataagtaa

Human Homologue TBLASTN with ORF1: CTP synthase (CTPS) (NM_001905.1)
40 Drosophila EST LD27370 (AA941993)

Annotated *Drosophila* genome genomic segment AE003532

Annotated *Drosophila* genome Complete gene candidate CG6854 - novel protein, possible CTP synthase?

Human homologue of Complete gene candidate

45

gi4503133 C33BD849A0044697 |ref|NP 001896.1| CTP

129

synthase; cytidine 5-prime triphosphate synthetase [Homo sapiens] (8.40E-217)

5

10

Putative function Enzyme important in the biosynthesis of phospholipids and

nucleic acids, and plays a key role in cell growth, development,

and

tumorigenesis. The region of the human gene is the location of

breakpoints involved in several tumor types

Confirmation by RNAi

Loss of G1 and G2/M peaks indicating fewer cycling cells

Example 35 (Category 4)

WO 01/72774

5 Line ID 225/27

Category Meiotic defects in testis: segregation defects

Reversion NR Map Position 90D

10 Rescue ID 2D2P

Rescue Sequence 1

Rescue ID 2D2E

15 Rescue Sequence 2

130

PCT/GB01/01297

- 20 CGCAAGAGCTAGCTCCACAGTGGATCCTAAGAGAACGGTCCCTGTGGACTCC
 ATCTAGCTAAGAGAAACGCACTTAGTTAGTTTCTATTTTTGGTTGTTTAAGTAC
 TGCTAGCTGCCTGCCAGTTGAGTGTCCGTCCAAAAACGGTGGTGGAAATGGGG
 GTGACCACTTCAAACATGAAAGCGAAATGTCCTGAGACCCTACAAAAACTAG
 AAATACGCGGGTGCACTGAGAGAAATTTTTTATTTCAAGTAAATTGGCAGAGG
- 25 CTACATTTTGAATGTTCACAATGAAAATTGCTGGGGAAGCTAGTGAACAACCA TTTCGCCATAATTTACACTATCTAAGCTTTTATTTTTAGCCACATGATATATGC ATGCA

30 Genomic hit, Accession No. AC008361

Associated ORF

Genscan ORF1 predicted sequences >20:36:39|GENSCAN_predicted_peptide_2|515_aa MSSTIRLQTSSCQCCKLYKYERHPNKPNLQPTPIPNYPCEILHIDIFALEKRLYLSCI

- DKFSKFAKLFHLQSKASVHLRETLVEALHYFTAPKVLVSDNERGLLCPTVLNYLR SLDIDLYYAPTQKSEVNGQVERFHSTFLEIYRCLKDELPTFKPVELVHIAVDRYNT SVHSVTNRKPADVFFDRSSRVNYQGLTDFRRQTLEDIKGLIEYKQIRGNMARNKN RDEPKSYGPGDEVFVANKQIKTKEKARFRCEKVQEDNKKNRNGKAAGGKGKTR RVARGAQIYQNWAICRNLFLFLSLACCRVCKVCDIVVEFRKGTNAVVNVQIREAI
- 40 SHVFHKEDIVIDVQESKEWCIWTDDQVQSPLPELENLWHELWIGPSHAYLIDQIVD LFENLLEKYNVQVVDVVRFNFLHRALVVVIISGIШIIMIIGVSGGQRTNAFSHHRS QRSAIGGDPQQKDSAVQQVQARSSDAFCQIPHRSPRFPGRSQLIPKPNREILRNASA TKNLLFRIRSQ
- 45 >20:36:39|GENSCAN_predicted_CDS_2|1548_bp atgtccagtacgatccgtctgcaaacttcctcatgtcagtgttgcaaacttctacaagtacgagagacaccctaacaaaccaaaccta

30

caacctacgccaattcctaactacccatgtgaaatacttcacatcgacatttttgcgctcgaaaaaaggttatacctaagttgtattgac a a atttag caa gitt g ccaa a ctttt ccatct g cag t caa a a g catct g t g cattt g c g a g a a cttt g c g a g a c c cta c a tta ctt c c a ctt a ctt a ctt c c a ctt a cttaccgccctaaggtcttggtttcggataacgagcgagggttgttatgccccacagtgctcaactatcttcggtctctagatatcgatct gtattatgctccaacccagaagaggaagtaaatggtcaagtcgagagattccactctacgttcctagaaatttatcgttgccttaaa 5 gatgagetecetacetteaaaceegttgagetggtacacatageagtggacegetacaacactteegtteaeteggtaaegaateg aaaaccagcagacgtttttttcgaccgctcgtcaagggtaaactatcagggtctgacagatttccggcggcagactttagaggacat caagggcttaattgagtataagcaaattagaggtaatatggclcggaataaaaatagggacgagccaaagtcttatgggccggga gatgaagtttttgttgcaaataagcaaataaaaacaaaggaaaaagcgaggttcagatgcgaaaaaggtacaggaagacaacaag aaaaatcgcaacggaaaagcggcgggtggaaaggggaaaactcgcagagtagcccgtggagctcagatttatcaaaactggg 10 ccaacgccgtcgtgaacgtgcagatccgtgaagctatcagccatgtgttccataaagaagacatagtcatcgatgtccaggagtcc aaggaatggtgtatttggaccgatgatcaggtgcagtcgcctctgccagaacttgagaatctgtggcatgaactgtggataggccc tagc catgcg tacctg at cgatcg at tagtcg at testing an aat at tagtcg at a aaa at tagtcg at a tagtcg at tagtcg at the tagtcg at tagtcg atca attice tecatege getete gtag te gtag te atte te geget at cate at tate at t15 aagaacaaatgccttttcacaccaccgatctcagcgatcagcgatcggcggcgaccctcaacaaaaagattcagcggtgcaaca ggtgcaggcacgatcttcggatgccttttgccagataccccaccgatctcccaggttcccagggcgcagccaacttattccgaagc caaatcgagaaattcttcgaaacgcgagtgccaccaaaaatttattgtttcgaattcgcagccagtga

Drosophila Gene Hit BLASTN with rescue sequence: couch potato (Z14974).
 Human Homologue BLASTX with couch potato: RBP-MS/type 2 (RNA binding motif family)(D84108)

Annotated *Drosophila* genome genomic segment AE003720
Annotated *Drosophila* genome Complete gene candidate CG18434 -couch potato RNA binding protein

Human homologue of Complete gene candidate 2224621 dbj|BAA20798| (AB002338) KIAA0340

(AB002338) KIAA0340 [Homo sapiens] (2e-19) and Ensembl predicted peptide Gene:ENSG00000070877 Clone:AC009710

35 Contig:AC009710.00004 (predicted unknown protein)

Putative function Possible RNA binding protein

132

Example 36 (Category 4)

Line ID 238/37

Category Meiotic defects in testis: segregation defects, multi-stage defects

(PI-02/17)

Reversion ?
Map Position 70D

Rescue ID I7E

10 Rescue Sequence

5

Genomic hit, Accession No. CSC:AC017664

25 Associated ORF

Genscan ORF1 predicted sequences >15:26:30|GENSCAN_predicted_peptide_1|1819_aa EMVQAKDPPSHYLSKLRTYLDPKASRSHRLYLFYFLCQKRKMVGESTSTQVLRD LEISLRTNHIEWVKEFLDDTNQGLDALVDYLSFRLQMMRHEQRLQGVLCASEERL NLTNGGDGGEIVMGNSSSVSPGGGGGLLSHGNSTGHGLANGTLDSRQQHTMSYG

- 30 FLRPTIADALDSPSLKRRSRHIAKLNMGAATDDIHVSIMCLRAIMNNKYGFNMVIQ HREAINCIALSLIHKSLRTKALVLELLAAICLVKGGHEIILGSFDNFKDVCQEKRRF QTLMEYFMNFEAFNIDFMVACMQFMNIVVHSVEDMNYRVHLQYEFTALGLDKY LERIRLTESEELKVQISAYLDNVFDVAALMEDSETKTSALERVQELEDQLEREIDR NSEFLYKYAELESESLTLKTEREQLAMIRQKLEEELTVMQRMLQHNEQELKKRDT
- 35 LLHTKNMELQTLSRSLPRSASSGDGSLANGGLMAGSTSGAASLTLPPPPPPMPASP TASSAAPPPPPPPAPPAPPPPGFSPLGSPSGSLASTAPSPPHAPPMLSSFQPPPPPVA GFMPAPDGAMTIKRKVPTKYKLPTLNWIALKPNQVRGTIFNELDDEKIFKQIDFNE FEERFKIGIGGALRNGSNGTEVDGSLQSSKRFKRPDNVSLLEHTRLRNIAISRRKLG MPIDDVIAAIHSLDLKKLSLENVELLQKMVPTDAEVKSYKEYIIERKDQQLLTEED
- 40 KFMLQLSRVERISSKLAIMNYMGNFVDSVHLISPQVQSIAGASTSLKQSRKFKAVL EIVLAFGNYLNSNKRGPAYGFKLQSLDTLIDTKSTDKRSSLLHYIVATIRAKFPELL NFESELYGTDKAASVALENVVADVQELEKGMDLVRKEAELRVKGAQTHILRDFL NNSEDKLKKIKSDLRHAQEAFKECVEYFGDSSRNADAAAFFALIVRFTRAFKQHD QENEQRLRLEKAAALAASKKENDQVLMRNKVNQKKQQEAVINELKSKAHSVRE
- 45 KKLLQQDEVYNGALEDILLGLKSEPYRRADAVRRSQRRRIDNNRLSRTLEEMDCL HENDLVKCALIADVLNLRSVHVTPVSSKDWEIIELSTEKISGSVLEQTRIVNSTQILI

10

VWINKSMQVALTVDRLKPHMNYGRIDHNTELVVAPNLYKGLTNGTSNGVIEENT KLSRSKTTAQVKDELTEKLTPLTHSSTVSNVKNTIQRNKRQDHMERLKKDLRRES SRSFEFRVIRGLWREQAQESDVFVNGKHLPEFFDLDLFYCMHTAADKDYYVRVR TVEDDIEDDLPETIHPSIELNANLMKLLGIKELERVVLRPKTTVVNFVEKIELFANK KTHYKIMENAFKRFVIERTQHKPMLFNQEEVVRLEDDLLVTVGILPEHFRYCVVD AQFLKESKIYAADLVRPVGEIIKEETPPTSPLSVQDLIQLPEYDKIVDQVVQELRMN LCLSADNSVMRQCNVLLAGASGTGKTVLVERILDQLSRKPDYCHFEFFHGSRSKG RKTESIQKDLRNIFTSCLQHAPAIVVLENLDVLAHAAGEQSSQDGEYYNRMADTV YQLIVQYTTNNAIAVIATVNELQTLNKRLSSPRGRHVFQTVARLPNLERADREIILR ELCSHINVAKDLDLVKFSNLTEGYRKCDLVQFVERAIFYAYRISKTQPLLTNDQLI ESLEHTNSYCLQGIQSNQRTGNDADANEMRVEELPGLESVVGVLEEVLMWPSRY PTIFNASPLRNQAGVLLYGPPGTGKTYLVSQLATSWNLRIISVKGPELLAKYIGQSE

15 >15:26:30|GENSCAN_predicted_CDS_1|5457_bp:

ENVRNLFNRARSARPCVLFFDEFDSLAPKRGHDSTGVTDRV

gaaatggtgcaggcaaaggatccgccctcacattacttgagtaaactgcgcacatatctggacccaaaggcatcaaggagtcatc ggctttatctcttcttactttctttgtcagaaacggaaaatggtcggcgagtccacgtccacccaggtgctccgcgatctggagatctcgctgcgcacgaaccacatcgagtgggtgaaggagttcctggatgacacgaaccagggtctggacgccctggtcgactatctcag cttccgactgcagatgatgcgacacgagcagcgccttcagggtgtcttgtgtgcctcggaggagcgtctgaatctcacaaacggc 20 gga cat gg cat a t gg cat a t gg cat a cat gg cat a cat a t g cat a cat gg cat cat gg cat a cat gg cat gg cat c $cattat {\tt gtgcctgcgagctatcatgaacaataagtatgggttcaacatggttatccagcatcgcgaggccatcaactgcattgccttg}$ agtet tate caca a a teget gag gac gaa age cet gg teet gg age cate t g te t gg ta a ag gg ag gac ac gaa ag te tate caca a a teget gag gac gac gag ag te tate caca a a teget gag ag gac gac gag ag te tate caca a a teget gag gac gac gag ag te tate caca a a teget gag gac gac gag ag te tate caca a teget gag ag te tate caca a temporary a temporar25 ggcctttaacatagattttatggttgcctgcatgcagttcatgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgttgtccactcggtggaggacatgaactacagggtgcactgaacatcgatgaacattta cag tac gag ttta cag cect gg gett gg at a a gtatet gg ag ga at teg at tac ag a at teg ag ag a act ga ag gag act ga ag ga act ga ag gag act ga ag ga act ga acat cag cct at ttg gacaac g tcttt gat g ttg ct g cct t g at g gag g at t ccg a gacaa aa aac tt cag ccct g gaac g a g ccaa g a caa g agcttgaggatcaacttgagcgagaaatagatcgtaactcagagttcctctataagtatgcggaattagagtccgagagtctaacgct30 acctccgccgccaatgcccgcctcgcctactgcaagttcagctgctcctccaccacctccgccgccagcaccaccggctccaccaccaccgccgggcttcagtccgctgggcagtccgagcggcagcctagcctcgacagcgccatcgccgccacatgccccgccc atgctaageteetteeaacegecacegeeteeagtggeeggetttatgeeegeteeegatggegecatgaceateaaaegeaagg 35 acgaaaagatcttcaagcaaatcgacttcaatgagtttgaggagcgcttcaagatcgggattggcggtgctttgcgcaatggtagcaatggtagcaatggcaatggtagcaatggtagcaatggcgattggcgaatggcgaatggcaatggtagcaatggtagcaatggtagcaatggtagcaatggcaaa atggaaccg aggtcg atgggtcg ctg cag to cag caa acgett caa gag gcccg a caatgtctcg ctg ctg cag cacacgag and the companion of the companion ofgttaagaaacattgcaatctcccgtcgcaagctgggtatgcccattgatgatgtcatcgccgccattcatagtctggacctgaagaa actttccctggagaacgtcgagctgctgcaaaaaatggtgcccacggatgccgaggtcaaatcctacaaggaatatatcatcgag 40 cg caaggac caa cag ctact caccgaag aagac aagtt tat g ctg cagtt g t cg cg t g t g c g c g t a t ct cg t c caag cta g c cag cg cg ca g c caag cta g cctttaagctgcaatcgctggacacgctgatcgatacaaaatccacagacaagcgatcgtcactgcttcactatattgtggccaccat acgggccaa atttccggagctgctgaacttcgagagcgagctgtatggaacagacaaggctgcatcggtggcactagagaatgt45 ggtggccgatgttcaggagcttgaaaagggcatggatctggtgcgcaaggaggccgagctgcgagtgaagggtgcccagacg

a aggagt g c g t t g agt a c t t t g g c g a at g c ag at g c g g t t c t t t t t t t t t g c g t t g at c g t t c a c g a g a g c g at g c g t t c a c g a g a g c g at g c g t t c a c g a g a g c g at g c g

gtacagecegeggatgctgtgcggcggtcgcagcgccggaggatcgacaataatcgtttatcgcgcaccctggaggaaatgg attgtctgcacgagaatgatctggtcaagtgtgcgctcatcgctgacgttctcaacctgcgcagggtccacgttacccccgtclcgt ccaaggactgggagatcatagaacttagcactgaaaagatatcgggcagtgtgctggaacaaactcgcatagtgaattcaacgca gatccttattgtttggattaataagtcgatgcaagttgcgctgacagtggatcgtctgaagccgcacatgaactacgggagaataga aactetecagaagtaaaaccaetgeecaggteaaggatgagtgaetgaaaagttaacaccgttgaeccatteetecaeggtgtee 10 aatgtgaaaatactattcagcgtaacaagcgtcaggatcacatggagcgtcttaaaaaggacttgcgccgcgaaagctcgcgta gcttcgaatttcgtgtcattcgaggtctatggcgggagcaggccaggagtcggatgtgtttgtgaacggaaagcatctgcctgag ttetttgatetagatetattetattgeatgeacacegeageegacaaggattaetatgtgagagtgegeacagtagaagaegatattg aggacgatctaccagaaaccattcatccatcgatcgaactaaatgccaatcttatgaagttgctgggtattaaggaattggaacgag tggttctaagacctaaaactaccgtagttaactttgtagaaaaaattgagctatttgccaacaagaagacgcactacaaaatcatgga 15 gaacgcatttaagcgatttgtgatagagagaactcagcacaagccgatgctcttcaaccaggaggaggtggtacggctggagga cgatttactggttactgttggaattttaccagaacactttcgttattgcgtggtggtcgacgcgcagtttctgaaggagtccaagatctacg cagcagatctggtgcgtccggttggcgagattattaaggaggagacgcctccgacatcgccactaagtgttcaggatctcatcca cagtgcaatgtcctactcgctggtgcctcgggaacgggtaaaacagttcttgtggagcgcattttggaccagctgtcacgcaagcc 20 taccag ctg cctg cag catgcccccg ccattgttgtgctagaaaacttggatgtactggcccacgctgctggagagcagtccagtcaggatggagagtactacaatcgcatggcggatactgtgtatcagttgattgttcagtataccaccaacaacgctattgcagtaatcg ccaccgtcaacgagttgcagaccctcaataagcgattgagctcaccaaggggaagacatgtcttccagactgttgctcgtctgccc ccaacctcacggagggctaccggaaatgtgatcttgttcagttcgtggagcgtgcaatattttatgcttatcgcataagcaagaccc 25 actggcaatgatgccgatgccaatgaaatgcgcgtcgaggagttgcctggcctggagtcagttgtgggagttctggaggaggtcc ttatgtggccatcaaggtatccaaccatttttaacgcctctccactgcgcaaccaggccggagtacttctatatgggccaccaggaa caggtaaaacctatctggtctctcagttggccacatcgtggaacctgcgcatcatttccgtcaagggtcctgagttgctcgccaaata tattggtcaaagcgaggaaaatgttcgaaacctgttcaatcgagctcgcagtgcccgaccatgtgtgcttttcttcgacgagtttgacatgtgccaaagcgaggaaaatgttcgaaacctgttcaatcgagctcgcagtgcccgaccatgtgtgcttttcttcgacgagtttgacatgtgccaaagcgaggaaaatgttcgaaacctgttcaatcgagctcgcagtgcccgaccatgtgtgcttttcttcgacgagtttgacatgtgcccgaccatgtgtgcctgacatgtgcccgaccatgtgtgcttttcttcgacgagtttgacatgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgtgcccgaccatgtgcccgaccatgtgtgcccgaccatgtgcccaccatgtgcccgaccatgtgccccgaccatgtgcccaccatgtgcccaccatgtgcccaccatgtgcccaccatgtgcccaccatgtgcccac30 agettggegeegaaaegtggteaegatteeaegggggteaecgategagtg

Drosophila Gene Hit recue sequence and TBLastn with ORF1: mRNA for I(3)70Da (AJ243811)

35 Human Homologue BLASTX with I(3)70Da: peroxisome biogenesis factor 1

(AF026086)

Drosophila EST LD43687 (AI512050)

Annotated *Drosophila* genome genomic segment AE003536

Annotated Drosophila genome Complete gene candidate CG6760 mRNA for l(3)70Da

- novel protein with homology to endoplasmic reticulum ATPases

45

135

ref|NP_000457.1|pPEX1| peroxisome biogenesis factor 1 >gi|2655141 (AF026086) (8e-80)

5

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Putative function

Putative member of the AAA protein family (ATPases associated with diverse cellular activities) including homologies to transitional endoplasmic reticulum atpases, and an E.coli membrane-bound AAA-type metalloprotease which degrades degrades sigma32, an alternative sigma factor for heat shock promoters

15 Confirmation by RNAi G2/M Slight loss of G1, increase in G2/M indicating arrest in

PCT/GB01/01297 WO 01/72774

136

238/44 Line ID

Meiotic defects in testis: segregation defects, multi-stage defects Category

(PI-02/18)

Reversion 5 Map Position

R 70D

F8E Rescue ID

Rescue Sequence

GTTCAAACGCACTTTTAAGGTGGCCTATCGGCCCATCAGGAGCAACTTTGTTC TGCTGCCGGATCAGTACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAGG 10 TCTTCGCTCCATTCAGATTAGATACGCCAAAGATTAATCCGGTCAACCATTCT GATTAGGACACGGGCTGCCTGAGCTTGCAGTACAATGGTCGGACGCACTACG CCTCCTGGGCGCACAAAAGGGCGGCGGCGCATTAAAGACACCGAGATTGG GATCAATGCCAGAGCGCCAAGGAGATCGGTAAGCCATTACTTAACGGCCGG ATGTGCATCGGTTGCCAATGTGCCGTAATATTGGACTCCGGCCATTTGCCCCG TACCTCGTACGCTAGCAGCACCCACTTACCCTTTCTTGCCGTATGTCTGCACGA GAATGATCTGGTCAAAGTGTGCGCT

Other results same as for line 238/37

20 .

Line ID 428/5

Meiotic defects in testis: cytokinesis defects, segregation defects Category

(seg-01/01)

Reversion 25

Map Position

70A

Rescue ID

G4E

Rescue Sequence

- GTTCAAACGCACTTTTAAGGTGGCCTATCGGCCCATCAGGGAGCAACTTTGTT 30 CTGCTGCCGGATCAGTACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAG GTCTTCGCTCCATTCAGATTAGATACGCCAAAGATTAATCCGGTCAACCATTC TGATTAGGACACGGGCTGCCTGAGCTTGCAGTACAATGGTCGGACGCACTACG CCTCCTGGGCGCACAAAAGGGCGGCGCGCGCATTAAAGACACCGAGATTGG
- GATCAATGCCAGAGCGGCCAAGGAGATCGGTAAGCCATTACTTAACGGCCGG ATGTGCATCGGTTGCCAATGTGCCGTAATATTGGACTCCGGCCATCTGCCCCG TACCTCGTACCTAGCAGCACCCACTTACCCTTTCTTGCCGTAGGTCTGCACGAA AATGATCTGGTCAAGTGTGCGCTCATCGCTGACATTCTCAACCTGCGCA
- Other results same as for line 238/37 40

137

Line ID 848/7

Category Mitotic defects in brain: cytokinesis defect. Meiotic defects in

testis: cytokinesis defect. Multi-stage defects

Polyploidy, no overcondensation

PI-01/10

Reversion R Map Position 70D1-2

Rescue ID G1E

10 Rescue Sequence 1

5

GGCCACCTTAAAAGTGCGTTTGAACATTCTCGTCGTGGGCGTGTGCGAATTTA GTACGCTCCTTGCTTTAAATCATTTTCGCACTAAACTTCTGCTCTCAGCGG AATTTACTTTTGCTTTATTAGAGATGGGAGCTCGCGCATCAGCTGAGCCGATA CTTGCGCAACAGGTGATACAGCTGATTAGAGATGGCCCTTTTCAACTGTTCCC

- 20 ATATCGATTTCCCTTCACTTTCGCTCCTCGTATACCATGCTGGGGTCTTATCAA ATTTATT

Rescue ID G1P

Rescue Sequence 2

CTCGTCCAA

25 AAGGTGGCCTATCGGCCCATCAGGAAGCAACTTTGTTCTGCTGCCGGATCAGT
ACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAGGTCTTCGCTCCATTCAA
ATTAGATACGCCAAAGATTAATCCGGTCAACCATTCTGATTAGGACACGGGCT
GCCTGAGCTTGCAGTACAATGGTCGGACGCACTACGCCTCCTGGGCGCCACAA
AAGGGCGGCGGCGGCATTAAAGACACCGAGATTGGGATCAATGCCACAGCGG
CCAAGGAGATCGGTAAGCCATTACTTAACGGCCGGATGTGCATCGGTTGCCAA
TGTGCCGTAATATTGGACTCCGGCCATCTGCCCCGTACCTCGTACGCTAGCAG
CACCCACTTACCCTTTCTTGCCGTAGGTCTGCACGAAAAATGATCTGGTCAAG
TGTGCGCCTCATCGCTGACGTTCTCAACCTGCACAGCGTCCACGTTACCCCCGT

35

Other results same as for line 238/37

138

Example 37 (Category 4)

Line ID 252/40

Category Meiotic defects in testis: segregation defects, abnormal spindles.

(Ab-03/30)

Reversion R Map Position 84E

Rescue ID A4B

10 Rescue Sequence 1

TACATGACTCTGCGATTTGACAAAAACAAAATTGAGTTTTGTCAAGAAAATCA ACTATTTTCTGTGTTTAAAAAACCGAAGCCAACAAATCCGACCAAAATGCCT GCCGAAAACTTGGAGGAGCAGGGTCTGGAGAAGAACCCGAACCTGGAGCTGG CCCAGACGAAGTTCCTGCTTACCCTGGCGGAATACAAGCAGGATGCGGCATTG

15 AAGGCGAAGCTTCTGGAGGCGATTCGCACGGAGAATATGGCCCCGTGGGTAC GAGCACATCCTGCTCCGGAACTCGGCTTGGACCCGTTAGACAAGGATCTTGCC TGGCGCCGAATTGAAGGAAAAACAATCGCGTTTAAGTTGGGAGCCA

Rescue ID A4E

20 Rescue Sequence 2

- 30 ATTGATTTTCCTGATAAAAATTTTCGCTTGGAAGCTACAGCATCGTCCACTGTC CATGTTTATATATCCTTATATTTGCCTATAAATATAT

Genomic hit, Accession No. AC006494

35 Associated ORF

Genscan: ORF1 predicted sequences >23:00:28|GENSCAN_predicted_peptide_2|389_aa MPAENLEEQGLEKNPNLELAQTKFLLTLAEYKQDAALKAKLLEAIRTENMAPWY EHICSELGWTVDKDLLARMKENNRVEVEQLDAAIEDAEKNLGEMEVREANLKKS EYLCRIGDKAAAETAFRKTYEKTVSLGHRLDIVFHLIRLGLFYLDHDLITRNIDKA

40 KYLIEEGGDWDRRNRLKVYQGVYSVAVRDFKAAATFFLDTVSTFTSYELMDYPT FVRYTVYVAMIALPRNELRDKVIKGSEIQEVLHGLPDVKQFLFSLYNCQYENFYV HLAGVEKQLRLDYLIHPHYRYYVREMRILGYTQLLESYRSLTLQYMAESFGVTVE YIDQELARFIAAGRLHAKVDRVGGIVETNRPDNKNWQYQATIKQGDLLLNRIQKL SRVINI

139

15

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Drosophila Gene Hit BLASTN with rescue sequence 1 and TBLASTN with ORF1: 26S

proteasome regulatory complex subunit p42A (AF145308).

Human Homologue BLASTX with ESTand TBLASTN with ORF1: Hypothetical

protein KIAA0107 (D14663).

20 Drosophila EST

several including GH17651 (AI387197)

Annotated Drosophila genome genomic segment AE003739

Annotated Drosophila genome Complete gene candidate CG5378 - Rpn7 19S

proteasome regulatory

particle, non-ATPase protein,

subunit S10aHuman

Homologue

30 Human homologue of Complete gene candidate gi7661914

8843E6684AE91ACD

[ref]NP_055629.1| KIAA0107 gene product [Homo sapiens]

(3.40E-149)

35 Putative function

component of the 19S proteasome regulatory particle

Confirmation by RNAi Marked decrease in G1 and G2/M indicating fewer cycling cells

140

Example 38 (Category 4)

Line ID 277/7

Category Mitotic defects in brain: anaphase defects

(weak, higher condensation, some polyploidy, fewer anaphases,

polyploids with monopolar spindles)

Reversion ?
Map Position 71B

Rescue ID B8E

10 Rescue Sequence

5

AGTCGGCGCATGCGGAGAGAGAATCGAAAGAGAAAGAGAAGCAAAGAGAGCGACATACAGCAAAAACAATTCAAAAAGAACTGGTGAAGAATACGAAAATAAGATTTTTTTAAGGAAGTCGCGCTTTGATCCGTATCCGTTTTAGCGTCCAAGATTTTTTTAAATCGGACCTATATTTTTGAGGTACAGTGAAGCTTTGATGCGCCA

15 GTCTTATATGAGTTAAAGTTTTAACGATTGAAAGACACCCCTGAGCTGCTCAT TATATTTCAATATTTATAAACAATCTTATATCAGAGCTTGAGAGACTTGCATGC GCCACAAAATTCCAATTCCAATTCCAATTCCGGAATAATTTCACAATACTC AATTAACATACGTATTTTATGTTCGTAATTTTTTAAAATTCCCAGATTCCCAC AATTGCCATAATAATCTCGATTATGTTATTATACTCTGAGAAGTAGGAGTGTG

20 TGCAAAGACCACAAACAAATCATTAGGGGCGT

Annotated *Drosophila* genome genomic segment AE003584 Annotated *Drosophila* genome Complete gene candidate CG15383 – novel

25 Human homologue of Complete gene candidate none

Putative function No homologies to indicate function

Confirmation by RNAi Slightly increased G1 decreased G2/M indicating arrst in G1

141

Example 39 (Category 4)

Line ID 284/4

Category Mitotic defects in brain: anaphase defects

(overcondensation, polyplody (with overcondensation), few

anaphases, metaphase with bipolar spindle)

Meiotic

Reversion NR Map Position 89B

10

15

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Rescue ID 2C6E

Rescue Sequence

25

Annotated *Drosophila* genome genomic segment AE003711

Annotated *Drosophila* genome Complete gene candidate CG4275 - mor transcription

factor involved in chromatin

remodelling

30

Human homologue of Complete gene candidate CG4275- 4507081

|ref|NP_003066.1|pSMARCC 2| SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2(aa)

35

Putative function Transcription factor, regulator of chromatin

40

Confirmation by RNAi Decrease in G1 and G2/M and increase in polyploidy

142

Example 40 (Category 4)

Line ID 407/8

Category Meiotic defects in testis: cytokinesis defects

Reversion ?

Map Position 64B1-2

Rescue ID A9E

Rescue Sequence

Genomic hit, Accession No. AC005814 64A6-64B6

Associated ORF

- 25 Genscan ORF1 predicted sequences >22:57:22|GENSCAN_predicted_peptide_2|524_aa MGRRKDKPRVIPEQDARICRAICLCQLTMVLSCVSIVYLSVAIYSPSLKAFKSGFEL DPVMCQTVDRQMPNNCPWASCGEWCLTKTSGFCPQIHSIVRRNGTDIQLNNCTR VTNTSCAMIDLSRLNKFNCNNGTACNNIRGVFNCSNGHCKNMSEFFLCHHKADG LTVNSQKDNTKLNGFFECHGVHCTKIKKPFSCDRYCSKITTTNVNTLIMHEDNLIA
- ADCENAVAFNQARGSEHGVRIEPFEFWKEDDGNLLTNCATVTRESDNRITATDCI NGTLLEHDTLPAPFMNFTQFWAIYENSTRSVDPEQRYLPNQANLTIYSWKKLFINL EGCVNTLRGECKDFVARYGNDGDNNTAQSRYQCYYNKDSNVEFVVARYDLDK VYRELLVSLIVPIVLFVISSISLCIITKSVKVGDDAKMRCVCAGDDSDNDGPFGPGL ANKQQDQMYDTDDDVVDLEHQAVDGQELSDHGLPLDNQELIGSTKSLIPISPVGE
- 35 SGTSDQIFDQDQEKATTCDVPEKPLVIL

>22:57:22|GENSCAN_predicted_CDS_2|1575_bp

(corresponds to CG15003)

Annotated *Drosophila* genome genomic segment AE003480
Annotated *Drosophila* genome Complete gene candidate CG15003- novel unknown

Human homologue of Complete gene candidate

none

20 Putative function No homologies to suggest function

Confirmation by RNAi Only wild type profiles observed

WO 01/72774

144

Example 41 (Category 4)

Line ID

422/28

Category

Meiotic defects in testis: segregation defects, multipolar spindles

5

(Mul-02/22)

Reversion

NR

Map Position

68E

Rescue ID

2I4E

10 Rescue Sequence

TCGTGGACCCTCAAAGNAACGGATTTCTCCAGTTTCTTCAAAGGGTTAATAAA
CTTTTCGCACGTTTCGCATTTTTATGCTCAATCCGGTTACAAAATGCTGATAAA
ACCACTTGAACTACACGTTTCCGTACTGATAAGGGCTTTTCTTCTTATCTGACC
TCTGGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCGTGATACGCC

TATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCA
CTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATT
CAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTT
TTTGCGGCATTTTGCCTTCCTGTTTTTTGCTCACCCAGAAACGCTGGTGAAAGTA

AAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCT
CAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCCGAA

Genomic hit, Accession No. CSC:AC014962

25

Annotated *Drosophila* genome genomic segment AE003543
Annotated *Drosophila* genome Complete gene candidate CG5684 (putative

transcription factor, human

PCT/GB01/01297

homolog

30

Human homologue of Complete gene candidate

1e-100 4758946

ref|NP_004770.1|pPOP2| POP2 (yeast homolog) >gi|4106061|gb|AAD02685| (AF053318) CCR4-associated

35

regulator of polymerase II

transcription

40

Putative function

Transcription factor

PCT/GB01/01297 WO 01/72774

145

Example 42 (Category 4)

Line ID

422/5

Category

Meiotic defects in testis: segregation defects, abnormal spindles

(Ab-04/26)

Reversion

5

15

Map Position

82D

Rescue ID

B9E

Rescue Sequence 1 10

ATTGGCTCTTGATGGACTACAACGCTACCAAAATGGGGCTTGAGTTGAATTAC CTGTTGGAAGACACAATGCCACCCACGATCAACAATTCGGCGGTAAACAGTG CCCTAAACAGACCCAGATACTCAGACTGATGTGTACCTTGCAGATCCGAGATC ATTTGCCGCGTGAAGTATGGAAACAACCTGCCGGATATACCATTTGATCTGAA

GTTTCTGCAGTACCCCTTCGACAGCCACCGCTTCGTGCAGTACAACCCAACGT CGCTAGAGCGTAACTTCAAGTATGACGTGCTGACGGAACACGATTTGGGTGTC ACGGTGGGACCTGATTAACCGGGAGCTCTATCAGGCCGACTCCATGACGCTGC TGGACCCGCCGATGAAAAACTGCTGGAGGAGGAGACTCTGACGCCCACAGAC

TCTGTGCGTTCGCGCCAGCATTCGAGGACGGTGTCATGGTTGCGCAAATCCGA 20 GT

Rescue ID B9B

Rescue Sequence 2

- GGCCAAATCTAGAAATCCTCAAATCTGCGCTTGGCAGTGTGACCGTACTTGAC CGGTACGATAATACCTCCGGTAAAAAAAAATACTATATTTCCGGGGGACTCAAA TGCAACATCCTCATCGTATATAACACAACATCTATTTGAATTTCATTTCCACAA CTAATATTATGGATAATGCTTTATTATCATTTTCCAAGTTAGCGATAAATCACC CCACAAGCTGAAAAATCAACGTTTAAAAACGATTGATATTTTTTTAATACTTT
- TTGGTTTTACTATTTGAATTTTTGTATACTTTTAGATTTTACTATTTTAATTTTTC 30 GTTTCTTCTAGCTGACTAACGGGTTAAAAAAGGATCCGTCGACCTGCAGATCT CTAGAAGCTTGCGTTGCTGGCGTTTTTTCCATAGGCTCCGCCCCCTGACGAGC ATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATA AAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCCGTGCGCTCTCCTGTTCCG
- ACCTGCCGCTTACCGGATACCTGTCCGCCTTCT 35

Genomic hit, Accession No. AC008189

Associated ORF

- Genscan ORF1 predicted sequences >15:53:24|GENSCAN_predicted_peptide_3|211 aa MRNANESSGKPKSKFVSNEFHALFSTICSIADSPAVSREKLKIDLAARKIPSASAPK GDSPLERFSRDLFTYLRSVCRWGRFSAALFTAELLIVGGIVSSNRTSESSETGNPLA NEPDPLYMKLVDPMVAGESPKRMIKDQKDVGLKSTSSSEELRKLPKTRGRQKRFI RNPNYVKANEFYDKMLSSEYVSKRYKDLPPPHPGFGADQPPA
- 45 >15:53:24|GENSCAN predicted CDS_3|636_bp

146

Corresponds to CG2503

10

Annotated *Drosophila* genome genomic segment AE003605
Annotated *Drosophila* genome Complete gene candidate CG2503 - novel possibly RNA binding

15 Human homologue of Complete gene candidate

3287674 AC005239 (AC005239) F23149_1(aa)

Putative function

Possible RNA binding protein

20

Confirmation by RNAi Almost no G1 and broadened G2/M indicating arrest in G2/M

Example 43 (Category 4)

Line ID

423/14

Category

Meiotic defects in testis: cytokinesis defects, abnormal spindles

5

Reversion

Ŕ

Map Position

67B1-10

(Ab-16/13)

Rescue ID

E9E

10 Rescue Sequence

- 20 CAATAATTGTAATTTATCCTTACAAAATGTTA

Genomic hit, Accession No. CSC:AC020214

Drosophila EST

several including LP12306 (AI297868)

25 Annotated *Drosophila* genome genomic segment AE003552 Annotated *Drosophila* genome Complete gene candidate CG3967 - novel

Human homologue of Complete gene candidate

none

30 Putative function

No homologies to indicate function

Confirmation by RNAi Only wild ty

Only wild type profiles observed

Example 44 (Category 4)

Line ID 427/5

Category Mitotic defects in brain: anaphase defects. Meiotic defects in testis:

segregation defects, abnormal spindles

(mitotic: Overcondensation, lagging chromosomes/less aligned

metaphase with bipolar spindles, Meiotic: Ab-06/20)

Reversion

Map Position 67B1-5

10 Rescue ID H4E

Rescue Sequence

5

15

GTACAGCCTGAAGTGATCGTTGTTGTTTGAATCGGTGCTATCGGCGGTTGCGC
TTTGTGGGCATCTTTATCCAATTTGCTATGCGCGCTTGTCCTTAAATTTTGAAC
TGTATTCCAAGGGTTGCTTTGGCGGCTATCGATAGTATCGGCATGGTTACATTT
TAGTTTTATAACAAGAATTTTACAGGTATTTTGATTATCTGAGCTTAGTTTTAA
GCAANAATATTATTGTTAAAAAATTTAAAAAAGTAAACAAGCTATTTTAACAAGC
ATTTAAACAAATAGTATTAATAATATAAAAAATATATCGATATGTGTTGCAAAT
GTTCGTTCCCTTAGTATTCTCTCATATTTATTTCAAATAAACTGTATAAAAATAT

CTGAAAAAGCGAACATATTTATTTAATTTCATCGCAGATATCGATATCACAGC
20 GCTGCTATCGATGGTGTCTGTCGCAGTGCCTATCGCTTACCCTGCCATCGCT
AACAAAAA

Genomic hit, Accession No. CSC:AC020120

25 Associated ORF

Genscan: ORF2 predicted sequences >22:06:07|GENSCAN_predicted_peptide_7|464_aa MPSEQHTNIKVAVRVRPYNVRELEQKQRSIIKVMDRSALLFDPDEEDDEFFFQGA KQPYRDITKRMNKKLTMEFDRVFDIDNSNQDLFEECTAPLVDAVLNGYNCSVFV YGATGAGKTFTMLGSEAHPGLTYLTMQDLFDKIQAQSDVRKFDVGVSYLEVYNE

- HVMNLLTKSGPLKLREDNNGVVVSGLCLTPIYSAEELLRMLMLGNSHRTQHPTD ANAESSRSHAIFQVHIRITERKTDTKRTVKLSMIDLAGSERAASTKGIGVRFKEGAS INKSLLALGNCINKLADGLKHIPYRDSNLTRILKDSLGGNCRTLMVANVSMSSLTY EDTYNTLKYASRAKKIRTTLKQNVLKSKMPTEFYVKKIDEVVAENERLKERNKA LEAKATQLERAGNSGFDPLELKTWYSKIDAVYAAARQLQEHVLGMRSKIKNINY
- 35 ROTLKKELEEFRKLMCVDQRVCQESF

>22:06:07|GENSCAN_predicted_CDS_7|1395_bp

WO 01/72774

149

PCT/GB01/01297

cataaacaagctagccgacggcttaaagcacatcccgtaccggactcgaacctgacacgcatcctgaaggactcgttgggcgg
aaattgtcgcacattgatggtggccaatgtctcgatgagctcactgacctatgaagatacctacaacacccttaagtacgctagccg
agctaagaagatacgcacgactctgaaacagaatgtcctcaagtccaagatgccaaccgagttctatgtgaagaagatcgacgag
gtggtagccgagaacgagcgactcaaagagcgcaacaaggcgetggaggccaaggccactcagttggagcgcggccggcaat
agtggattcgatccgctggagcttaagacgtggtacagcaagatagacgctgtatatgcggccgcccggcagcttcaggagcac
gtccttggtatgcgtagcaagatcaagaacatcaactaccggcagacactgaaaaaaagaactggaggagttcaggaagctgatgt
gtgtcgaccagcgagtgtgccaggaagtttttaa

Drosophila Gene Hit TBLASTN with ORF2: kinesin like protein 67a (U89264)

Human Homologue (AF041853)

Drosophila EST GH22018 (AI402731)

Annotated *Drosophila* genome genomic segment AE003552

Annotated *Drosophila* genome Complete gene candidate CG10923 Klp67a - motor protein

| | Human homologue of Complete gene candidate | 2e-58 4758646 kinesin family protein 3B |
|----|--|--|
| 20 | | >gi 3913958 sp O15066 KF3B HUMAN KINESIN-LIKE |
| | | PROTEIN KIF3B and also |
| | | predicted peptide |
| | | ENSP00000166696 |
| 25 | | Gene:ENSG00000073652 |
| | | Clone:AC015936 |
| | | Contig:AC015936.00023 |
| | | 6.70E-91 (predicted kinesin?: ENST00000166696) |
| | | |

Putative function

motor protein involved in cytoskeleton organization and biogenesis

35

30

Confirmation by RNAi Almost no G1 and broadened G2/M indicating arrest in G2/M

150

Example 45 (Category 4)

Line ID 442/3

Category Meiotic defects in testis: segregation defects.

5 Reversion ?

Map Position 70D4-7

Rescue ID H7E

Rescue Sequence

20 Genomic hit, Accession No. CSC:AC017664

Drosophila EST CK02287 (AA141680)

Annotated *Drosophila* genome genomic segment AE003536

Annotated *Drosophila* genome Complete gene candidate CG6650 - novel transacylase like

Human homologue of Complete gene candidate none

30 Putative function Transacylase

Confirmation by RNAi Marked increase in G1 indicating arrest in G1

151

Line ID 473/22

Category Meiotic defects in testis: no division

(no meiosis)

Reversion R

5 Map Position 70A1-5

Rescue ID 2B7E

Rescue Sequence 1

20

Genomic hit, Accession No. CSC:AC017664

Drosophila EST LD47104 (AI515336), SD03663 (AI532240)

For other results see line 442/3

25

Line ID 670/6

Category Meiotic defects in testis: segregation defects, abnormal spindles

(Ab-12/48)

30 Reversion '

Map Position 70C

Rescue ID H7E

Rescue Sequence

45 Genomic hit, Accession No. CSC:AC017664

Drosophila EST CK02287 (AA141680)

For other results see line 442/3

152

Example 46 (Category 4)

Line ID 460/20

Category Meiotic defects in testis: segregation defects, multipolar spindles

(mitotic: High polyploids, no diploids, higher mitotic index

Meiotic: Mul-02/59)

Reversion NR Map Position 78A1-4

10 Rescue ID 2B8E

Rescue Sequence

ACCTTTAAG

25

30

35

15

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5

Genomic hit, Accession No. CSC:AC020460

Annotated Drosophila genome genomic segment AE003592

Annotated Drosophila genome Complete gene candidate CG10588 - novel gene with homology to proteases

Human homologue of Complete gene candidate

2e-74 4505453

ref|NP_002516.1|pNRD1| nardilysin (N-arginine dibasic

convertase)

>gi|2462488|emb|CAA6369

Putative function Novel protease

40

Confirmation by RNAi Marked increase in G1 indicating arrest in G1

Example 47 (Category 4)

Line ID

WO 01/72774

477/16

Category

Meiotic defects in testis: segregation defect.

153

PCT/GB01/01297

Reversion

NR?

Map Position

90C5-10

Rescue ID

C₃E

Rescue Sequence 1

CTGTGGACGGTCGTCAATGCGTGAATATTCTTCTATGTGTAAGTGGTGTGCGT 10 GTATGTAGATTTCTGGTTAAGAAAAGCCCCAAAAACCAAAGCGCCCCGCAAA ATATATATTGAGTCTTCTTGGCCCAACAACAAATCTGCCGCCGGACTTTCGCC GGAGGGCGAGTGAAAAATTCAGTTTCTCTCCTCTCGACGATGCACTTTGGAGG CTGTGTGAGTGTGTGCGAGTGAGTGCGTGTGTGTATACATATGCAAATGAT TGGATGTCGAATCCTTGCATCATCATCATCTTCATAAACACTTGGCGAAAAAC 15 CGCAGGAAAACGCAAGCAGCCGAACAAAAAAAAGAGAGCCTCTCAAGACAAC AAAATAAGTTAAACCAATTGGTGAAGATGATGCCCC

20

Rescue ID C₃P

Rescue Sequence 2

GTCCACAGACTGGCTATATATACTAAAAACGAACTCGCGTGAGAAGACAGGG ACAGGGCAGCAAACTCGGTATACGAACGGAACGAAATGAAACGATTCAAGTA 25 TCGATGGTTTTTCGCCAGGCTGGGCGCTGCCAAAACGCTGATACGGCGGCCAC AATCACACGCGGCTAATCGCCAGTTGGGCCCTGCACAGGCTGCACATACTTTT CACTATTAATGCGCTGTATTTCACTTATTTTTCGAACAAATTCGCAGCATGACG TACGTACGAGTATTTGGCACTGCGATACATTATCGGTGCTCGTTTCGATAGCCC CCGATAGCTCTAGCACGAAATTTTATCGCTTTATCCATATTTTATACTATTTTT ATTTATTGGACTTCAATGAATATTTAATTTACGTCTGGGTCGCTTTTTAAATAT ATATGGTAATCAATAGCTGGCGAATTAGCGATATTTGAGTGTGACGCAAAAAT GAGTTGCATCGATATCGATTTCTCGCTACTCTGGGACGCCATCTTTATTGCGG

35

Genomic hit, Accession No. AC007810

Associated ORF

Genscan ORF1 predicted sequences >17:48:58|GENSCAN predicted peptide 2|349 aa MSRILFILLLLIVTQLSELQAAAFSVRQNRFDEVPDLQTPAPLATSTESSKKPEKAT 40 SGLLKKCLPCSDGIRCVPQIQCPAHVRMESHEKPQICDLPAGKFGYCCETGQNHT APKPETSPKERRSGFPTILSPAVLDEARRNFEHLMHGVAQIPVRRGFPDFAHGLVF HSTAKDDLHNFAISNSAIEQVMTTQLFGKKEQVPVEDFITNNVPIKFTETPLAHHC QPPPVCGNIRSVYRSMDGTCNNPEPQRSLWGAAGQPMERMLPPAYEDVPSASPA

AICSYIYGIASRLAPVSVVNCCTFAWQLDWTTGMASGECVCVECMPAEWRLGQC 45 **PLLHEASSEMSRLLAKS**

154

>17:48:58|GENSCAN predicted CDS 2|1050 bp

Drosophila Gene Hit rescue sequence: eyelid/osa (AF053091)

Human Homologue BLASTX with eyelid: KIAA1235 protein (AB033061) Brain protein 120 (AB001895)

20 Drosophila EST several including LD04852 (AA201670), LD24466

Annotated Drosophila genome genomic segment
AE003718

Annotated Drosophila genome Complete gene candidate CG7467 - osa DNA binding putatively involved in DNA packaging

Human homologue of Complete gene candidate

CG7467 - 7e-25 2588991
dbj|BAA23269| (AB001895)

dbj|BAA23269| (AB001895)
B120 [Homo sapiens] and
O14497 SWI/SNFRELATED, MATRIXASSOCIATED, ACTINDEPENDENT REGULATOR
OF

35 CHROMATIN SUBFAMILY F MEMBER 1 3e-67

40 Putative function transcriptional regulator

Confirmation by RNAi Only wild type profiles observed

WO 01/72774

155

Example 48 (Category 4)

Line ID

Category

Meiotic defects in testis: segregation defects, abnormal spindles

5

(meiotic: Ab-08/42)

Reversion Map Position NR 65E4-7

2C1E

Rescue ID 10 Rescue Sequence

CCTITGCTACCGTTTCGCAGAACAGGTGTATCGGTTTTCAAGGCAACTGTGATT TTTTAACTCAACATTCTATATCGAAAACTTGTAGAGGTCGGAATTTTTCTTGAG CGCCTAAAAGTGTGCAGTGAAATCATTTAATCCACTTCCGGTTGCAAAACAGG

AATCACACATATGAAGTGATTAAAAATCATAGAAGGTTTGACACCTTCAAATA TTTAATGGCCTACAAATCTGTTGAATATCCGTTGAATACACTTTTCCAGGGTGT GTCCTAGTCGGCTCCTCTTTGTTACCCCAGTTTGCTGGTCTTCTTAGCCGCACA CCAGTTTATCGCTGTTTTGCCTTTGCGCTTTTCATTCATAAACAAAAAAACAATG

TTATTGTTATTGCGGTGGCTGTAGATGTAAATGTAAATGTAGATGTAGAGGCT 20 **GCTTCTTGGG**

Genomic hit, Accession No. CSC:AC018039

Associated ORF 25

Genscan ORF1 predicted sequences >19:35:36|GENSCAN_predicted_peptide_6|190 aa MVSEQFNAAAEKVKSLTKRPSDDEFLQLYALFKQASVGDNDTAKPGLLDLKGKA KWEAWNKOKGKSSEAAOOEYITFVEGLVAKYDNGMHKQEPNTCQARNATRFR KSSECSLDQNTYTSSVTVIPAFHEGPKNSTASWPRIYRCYQRNQQAANCKWANTN SVCGKPHGKQSRRIIFAEFLAGHTVQILG

>19:35:36|GENSCAN predicted CDS 6|573_bp

atggtttccgagcaattcaacgccgccgagaaggtgaagagcctgaccaagcgtcccagtgatgacgagttcctgcagctg tacgccctgttcaagcaggccagcgttggtgacaacgacaccgccaagccgggtctcctggacctgaagggcaaggccaagtg caagtatgacaatggaatgcacaaacaagaaccaaacacttgccaagcacgcaatgcgactcggtttcggaaaagctcggaatg ctcgctggatcagaatacgtatacgtccagtgtgacggttatacctgcattccacgaaggtccaaagaactcgacggcaagttggccaagaatttaccggtgctatcagcggaaccaacaagcggccaactgcaagtgggcaaacacaaatagcgtttgcgggaaaccc

40

35

30

Drosophila Gene Hit rescue sequence: melt (S144114) P element insertion site (AF174669), TBLASTN with ORF1: diazepam binding inhibitor (DBI) (U04823) and melted (AF205831)

Annotated Drosophila genome genomic segment AE003560 45 Annotated Drosophila genome Complete gene candidate CG8624 melt - putative signal

156

| | | transduction protein | |
|----|------------------------|--|--|
| | | CG8631 msl-3 - acyl-CoA- binding | |
| | | protein/diazapam binding | |
| 5 | | inhibitor | |
| , | Human homologue of Com | plete gene candidate CG8624- predicted gene ENSP00000065899 | |
| | | Gene: ENSG00000055889 | |
| | | Clone: AC015904 Contig: AC015904.00014 | |
| 10 | | 1.70E-15 (unknown predicted | |
| | | gene 1: ENST00000065899 | |
| | | and AK022666 Homo sapiens | |
| | | cDNA FLJ12604 fis 2e-29 | |
| 15 | | | |
| ., | | CG8631- gi5803104 | |
| | | 0C85AE40FDF874CD | |
| | | ref NP_006791.1 male- | |
| | | specific lethal-3 (Drosophila)- | |
| 20 | | like 1 [Homo sapiens] (1.70E- 36) and Ensembl predicted | |
| | | peptide ENSP0000006617 | |
| | | Gene:ENSG0000005302 | |
| | | Clone: AC004554 | |
| 25 | | Contig:AC004554.00001 | |
| | | 8.70E-19 (unknown predicted gene 1: ENST00000006617 | |
| | | gene 1. ENST0000000017 | |
| | | | |
| 30 | | | |
| 50 | Putative function | CG8624: putative signal transduction protein | |
| | 2 40000, 2 50000 5000 | CG8631:acyl-CoA-binding protein/diazapam binding | |
| | inhibitor | | |
| | | | |
| 35 | | | |
| | Confirmation by RNAi | CG8624: reduced G1 and G2/M Indicating fewer cycling cells, CG8631: Increased G1 to G2/M ratio indicating arrest in G1 | |

157

Example 49 (Category 4)

Line 1D 523/19

Category Female sterile. Meiotic defects in testis: cytokinesis defects,

segregation defects (Mitotic: Less condensed chromosomes, nuclear

bridges, Meiotic: Seg-01/02

Reversion R Map Position 75C1-4

0 Rescue ID 2B4E

Rescue Sequence

5

- 15 AAGAATGAATGAAGCCAATGAATTTTCAATAGTAATTCAGAGTGCTTAAAATT
 CTTCATGTTGTCATTGAGTAAAATGAGTTCGGACAGCGCGAAGGTAAGTCGAA
 GTTTGTGTTTTATTATGTTTATTTTTTATTATTATGTACACTAGTCGGCATACTTT
 TGCGTGCGTCTTATACGTGTGCGTCTTATTTAACAATATTGTAAAATAAAATAT
 ATAAATTATTTGTTATATGCGTAGGGGCCTTTATTTTGTGTATTGATAGTCTTTT
- 25 Genomic hit, Accession No. AC007691

Annotated *Drosophila* genome genomic segment AE003520 Annotated *Drosophila* genome Complete gene candidate CG4306 – novel

30 Human homologue of Complete gene candidate 4e-25 3242764 (AC005154)

similar to protein U28928 (PID:g861306) [Homo

sapiens]

35

Putative function No homologies to indicate function

Confirmation by RNAi Only wild type profile observed

Example 50 (Category 4)

Line ID

666/19

Category

Mitotic defects in brain: anaphase defects

5

(weak, overcondensation, aneuploidy, lagging chromosomes,

metaphase with bipolar spindle)

Reversion

NR

Map Position

64E1-5

10 Rescue ID

I9E

Rescue Sequence

CCCTCGTCTACGTCGAAATTCTGGATGCTTCTCGGATTTAGGGTTGTATCTCGA AAACGTTTGACTGCGAATGTCAATATCGATATGCTAACCGATAGCTGTCGATG TTITAAACACAGTGCACTGTTTTTAAATCGCTCCCCATTTATATATTTTGTGC

- 15 NTGCTTTTGGCGGTNNTTTTCTTTATATGCTTCTTATGCTTTTACGATTATTATT AGCGCTTATTTGATTGCAAATGCCAAGGAAAGCGTGACTGTGATGGCGAAATG CGGAAAGTACTCCTTAAATCTCATATATCGCATAAAACTATCGGTTCTGGAAT GTTTCGTGAAGTCTGCGAAGATAGAGATCGATCTATTTTGAGGATACATTTG TTAATATTATAAGGGATTCTTCTACAGGGGTCAGATTGCTTAAAAACCACAG
- 25 GGGCCT

Genomic hit, Accession No. CSC:AC014815

Associated ORF

- 30 Genscan ORF1 predicted sequences >17:46:43|GENSCAN_predicted_peptide_1|334_aa MGKDFYKILGLERKASDDEIKKAYRKLALKYHPDKNKSPQAEERFKEIAEAYEVL SDKKKRDIFDNYGEDGLKGGQPGPDGGGQPGAYTYQFHGDPRATFAQFFGSSDP FGAFFTGGDNMFSGGQGGNTNEIFWNIGGDDMFAFNAQAPSRKRQQDPPIEHDLF VSLEEVDKGCIKKMKISRMATGSNGPYKEEKVLRITVKPGWKAGTKITFPQEGDS
- 35 APNKTPADIVFIIRDKPHSLFKREGIDLKYTAQISLKQALCGALVSVPTLQGSRIQV NPNHEIIKPTTTRRINGLGLPVPKEPSRRGDLIVSFDIKFPDTLAPSLQNQLSELLPN

>17:46:43|GENSCAN predicted_CDS_1|1005_bp

gacgccagctgacatcgtcttcatcattcgcgacaaaccgcattcgctgttcaaacgcgagggaatcgatctaaagtatacagcccagatcagtctgaagcagggcattgtgcggagcactggttagtgtgcccacgctgcagggcagcaggatacaggtgaatccgaaccacgaggatcatcagggccatcgaggccggatcaacggatcggctggtctgccggtgcccaaggagccatcgaggcgggggatctgatcgtctcttcgacattaagtttcccgacacactggcacccagtctgcagaatcagctgccgagctgctgcccaactag

5
Drasanhila Gene Hit

WO 01/72774

Drosophila Gene Hit rescue sequence: fasciclin I (FasI) (M32311) TBLASTN with

ORF1: DnaJ homolog (DROJ1) (U34904)

Human Homologue

TBLASTN with ORF1: DnaJ-like heat shock protein 40 (HLJ1)

(U40992.2)

10

Annotated Drosophila genome genomic segment

AE003565

Annotated Drosophila genome Complete gene candidate CG10578 - DnaJ-1 a

chaperone putatively involved in protein folding. Stimulates

PCT/GB01/01297

activity of HSP70

15

Human homologue of Complete gene candidate

8e-94 1706473 P25685

DNJ1_HUMAN DNAJ PROTEIN HOMOLOG 1 (HDJ-1) (HEAT SHOCK

20

PROTEIN 40) (HSP40)

Putative function

Chaperone involved in protein folding

25

Confirmation by RNAi Almost no G1 peak, increased G2/M indicating G2/M arrest

160

Example 51 (Category 4)

Line ID 714/11

Category Meiotic defects in testis: cytokinesis defects, abnormal spindles

(Ab-01/04)

Reversion ?

Map Position 66A10-15

Rescue ID 2A4E

10 Rescue Sequence

AACCAGAACGAAACTCCAATGCAGTTTCATTTTGTCAGTTTAATCATTAAACA
AAGAACTGCGCAACCGATCGCAACTAGCTCGTGGACTCTTGTTCTCCCAATAA
TTGGTATGTTTTCCATTTTGCGTTAACATGGAAAATGTGTGAAAAGCTTTTTCC
CCCTCCAAAAGAAGCGTACTGAACTAAGCTTTCGGTGGTTAGTAATAGTAGTC
GTTATATCTTATTTTTCTTATTTACGTGCAGCTGCAATCATTGGCTGCGTCACTT
TGGCGTCAGCTATAAACTGGTGGATCAACTCGGCGGCCTCCAAAAGCTGCGCA
TCTGCTCCAGACACTTTAGCCAACGCCAGGAGATGGCCAAAACCCGCATCAA
GATGACGCCGCTGCGCAAGTCCTCCTCCAAGGGCATTGTGCTACCCATTA

ATGCCGCTGGAGGGTCGTCATTGCAGGCGCCTTAGCACGAGGAGGAGGTGC

20 A

15

5

Genomic hit, Accession No. AC012390

Associated ORF

- 25 Genscan ORF1 predicted sequences >19:47:45|GENSCAN_predicted_peptide_2|711_aa MRSHQAVGNLLLAADEALPAVQSASVYVVWMAEQPLSPGQSYDIKIADSPSVSS KSITDNGADVQWFAFEHSQYYQGVQQMFLSALERIDSEFLITLIKRCPYHVDSLVQ LSEVCKMTEDFSLASELLERALLLLESSLHINFSLTSGNCRLDYRRQENRSFYIVLF KHAQYLEERACSRTAFEISKLLLSLQPDTDPLAMILPNQPDQCTGNMTQLQQAGK
- IRKRSEKQFPIGTEPRGTDALRFTLQTLASAGRDITWNIKRLQGSRVTGAAQGYLI
 DKKTAVQYKITIIAHLKDPNIDQLFDSSGDGKADLHGSTPDWGCQAMMADAISR
 YKEGNPVFYYTWTPYWVSNELKPGKDVVWLQVPFSALPGDKNADTKLPNAGGI
 EGLIADEEVQVLDALCDAPCVGVSHSCRLLDGNRRGNNELRLFIPGKSQFGVADG
 CADKQSVMEYHAAKTGHTKFSESEEEKKALTEEEKKAQLALIEEKLKQKRIEREE
- 35 REKIEALQREKNRIKSGKDMTEAKRRMEELEMKKIVEQRKREKDEEKAARDRVK AQIEADKAARKAREQKELGNAEPAPSVSSTTVSSPPAGVKSPPRDYTETRIQGASA ILAAAAPYYQPPAVPQDVQPDRPIGYGAFGVVCGSHISGWHCSAGHYEDGNENFE CLKTFSTSDRIGCEWRWAAATVLAATCISPNGRCGHYKRVRRIKTNITTT
- 40 >19:47:45|GENSCAN_predicted_CDS_2|2136_bp
 - atgagatcgcatcaagccgttggcaatctgctgctggcggcagacgaagcgttaccggcggtgcagagcgcgtcggtgtatgtg gtatggatggcggaacagccgctttctccagggcagagttacgacatcaaaattgccgactctccatcggtgtcctccaagtctatc acagataatggagcggacgttcaatggtttgcctttgagcatagccaatactaccagggagtgcagcaaatgttcctttctgctctcgagcgattgactcggaatttctgatcacacttatcaaacgctgcccctatcatgtcgactccttggttcaactcagcgaagtatgcaa
- 45 gatgaccgaagacttttccttggcetccgaactgettgagegegecetteteettetggaategtegetgeacateaacttcagtttga cgtcgggcaactgccgactggactaccggagaaaaccgatccttctacategtgctgttcaagcacgcgcagtacctgg

161

aggaacgagcttgcagccgcaccgccttcgagatctccaaactgctcctgagtcttcagccagacacagatcctcttgccatgatt ctaccaaatcagccggatcaatgtaccggcaatatgacgcagctgcagcaggcgggcaaaatccgtaagcgctcagaaaagca gtttccgatcggtactgaaccgcgggtactgacgcgttgcgcttcaccctgcagacactggcgtctgccggtcgcgacatcacct ggaatataaagcgtctgcaaggttcccgtgttaccggcgcgcccagggttacctcatcgataagaaaaccgccgtccagtacaa a at caccat categorica tet gaa a agatee gaa ta tegacea act gttegat te a ageggega eggaa aa ageggat tt acae gg ta agatee gaa ta tegacea act gatee ageggaa ageggaa ageggaa ta ageggaa ta tegacea act gatee ageggaa ageggaa ageggaa ta ageggaa ta ageggaa agegga5 gtaccccagactggggctgccaagctatgatggccgacgccatcagtcgctacaaagagggcaacccggtgttttattacacctg gacgccgtactgggtgagtaacgaactgaagccgggcaaagatgtcgtctggttgcaggtgccgttctccgcactgccgggcga taaaaacecceataccaaactecceaateccegtegcatceaagectcategcceatgaagaagtccaggtcctcgatgccct ttgtgatgcgccgtgtgttggtgtctcccactcgtgccgactccttgatggcaatcgccgagggaataatgaactgcggctctttatt cccggcaaatcccagtttggagtagctgatggatgtgcagacaagcagagtgttatggagtaccatgccgccaaaaccggtcac 10 accaaattctccgaatcggaggagaaaagaaggcgctcaccgaggaggagaagaaggcccagctggccctcatcgaggag aagctcaagcagaaacgcatcgaacgcgaggagcgcgagaaaatcgaagccctgcagcgggaaaagaatcgcatcaagtcc ggcaaggacatgaccgaggccaagcggcgcatggaggagttggagatgaagaagatcgttgagcagcgcaagcgcgaaaa ggacgaggagaaggcggcccgcgatcgggtaaaggctcaaattgaggcggacaaggcagcacgcaaggctagagaacaaa 15 gagaciacaccgaaacccgcatccagggcgccagcgcaatcttggccgcagcggctccctactatcaaccgccggctgttccc caggat gtt cag ceggat cette tegget at egg age at teggag tegget teggeg tegge at teggag tegge at tegg at tegge at tegg at tgggcaltatgaagatggtaatgaaaatttcgagtgcctcaagacattttcgacttctgaccgcattggctgcgaatggagatgggcg gcagcaactettcttgccgcaacctgcattagcccgaacggccgttgcgggcattataaacgcgtacgtcgtcgcattaaaacaaa 20 cataacaactacgtga

Drosophila Gene Hit rescue sequence and BLASTX with EST: BIP1 (Y14998),

BLASTX with genomic sequence matches BIP.

Human Homologue BLASTX with BIP1: alanine:glyoxylate aminotransferase

(X53414)?

Drosophila EST GM04749 (AA695904), GM13608 (AA803601)

Annotated *Drosophila* genome genomic segment AE003556

Annotated *Drosophila* genome Complete gene candidate CG7574 - bip1 unknown function

CG13681 - unknown

Human homologue of Complete gene candidate none

Putative function

25

30

35

no homologies to indicate functions, Drosophila Bip1 interacts with transcriptional activator Bric-a-brac which is required for ovariole formation

40 Confirmation by RNAi Both show reduction in G1 and G2/M iondicating fewer cycling cells

PCT/GB01/01297

Example 52 (Category 4)

Line ID

5

763/4

Category

WO 01/72774

Meiotic defects in testis: segregation defects

(overcondensation, fewer anaphases)

Reversion

R

Map Position

90F

Rescue ID

2F5E-1

10 Rescue Sequence

Genomic hit, Accession No. AC006495

20

15

Associated ORF

Genscan ORF1 predicted sequences >22:47:02|GENSCAN_predicted_peptide_3|283_aa
MTERENNVYKAKLAEQAERYDEMVEAMKKVASMDVELTVEERNLLSVAYKNVI
GARRASWRIITSIEQKEENKGAEEKLEMIKTYRGQVEKELRDICSDILNVLEKHLIP
CATSGESKVFYYKMKGDYHRYLAEFATGSDRKDAAENSLIAYKAASDIAMNDLP
PTHPIRLGLALNFSVFYYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIM
QLLRDNLTLWTSDMQAEEIPIPKLPDRQSKTTLIFSPRSQVNPKILHKNNTIIGRVIC
SVFA

- 30 >22:47:02|GENSCAN predicted CDS_3|852_bp
 - atgactgagcgcgagaacaatgtgtacaaggcaaagctggccgaacaggccgagcgctacgacgaaatggtggaggccatga agaaggtcgcctccatggacgtagagctgaccgtcgaggagcgaaatctgctgtcggtggcgtacaagaatgtgattggagcac gccgtgcctcgtggcgcatcatcacctcgatcgaacagaaggaggagaaacaaggggggccgaggagaaattggagatgatcaa aacctaccgcggacaaggtggagaaggagctgcgcgacaatctgctcggatatactgaacgtgctcgagaagcatctcattccatg
- 40 cattgatttttagcccccgaagtcaagtaaacccaaagattctccacaagaacaacaccatcatcggcagagttatctgtagcgtgtt tgcgtga

Drosophila Gene Hit rescue sequence: 14-3-3 epsilon isoform gene (U84898)

TBLASTN with ORF1: 14-3-3.

45 Human Homologue TBLASTN with ORF1 and BLASTX with 14-3-3: epsilon isoform

14-3-3 protein (U43430.1)

| Annotated Drosophi | ila genome genomic segment | AE003721 |
|---------------------|--|--|
| Annotated Drosophi | ila genome Complete gene candidat | e CG8045 complex gene |
| • | | appears to encode 3 things: |
| | | Transcript: CT24102 unknown |
| | | Transcript CT24072: |
| | | transcription factor RNA |
| | | polymerase II transcription |
| | | factor, |
| | | Transcript: CT24092: |
| | | diacylglycerol- |
| | | activated/phosholipid |
| | | dependent protein kinase C |
| | | inhibitor /14-3-3 protein |
| | | epsilon (suppresspr of ras) |
| | | |
| Human hamalagua | of Complete gene candidate | CT24092: e-119 |
| Human nontologue | | ND 000762 Hammaina 2 |
| Human nontologue | | NP_006752.1 tyrosine 3- |
| man nontologue | | monooxygenase/tryptophan 5- |
| | | monooxygenase/tryptophan 5- monooxygenase activation |
| | | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; |
| | | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo |
| | | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; |
| | According factor on 14.2.2 mosts | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo sapiens |
| . Putative function | transcription factor, or 14-3-3 protein | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo sapiens |
| | transcription factor, or 14-3-3 protein phosphatases | monooxygenase/tryptophan 5- monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo sapiens |
| | phosphatases | monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo sapiens |
| | Annotated Drosoph | Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidat Human homologue of Complete gene candidate |

Example 53 (Category 4)

Line ID

951/8

Category

WO 01/72774

Mitotic defects in brain:

5

45

(some overcondensation, anaphase bridge, metaphase with

PCT/GB01/01297

swollen chromosome and bipolar spindle)

Reversion

NR 73D

Map Position

10 Rescue ID

2E8S

Rescue Sequence

- 25 Genomic hit, Accession No. CSC:AC015272

Associated ORF

Genscan ORF1 predicted sequences

- >23:03:05|GENSCAN_predicted_peptide_1|602_aaMGFDMATRFMDILKLTFKPFKTN

 YTEEKYFNDKLRSSKNIERRYILDVGFRGPTAVTYNPIWVISFKYEQRKLSTAIYSV
 IKTKSGPVRGVKRNTIWGGSYFSFEKIPFAKPPVGDLRFKAPEAVEPWDQELDCTS
 PADKPLQTHMFFRKYAGSEDCLYLNVYVKDLQPDKLRPVMVWIYGGGYQVGEA
 SRGLDVVIVTVAYRLGALGFLSLDDPQLNVPGNAGLKDQIMALRWVQQNIEAFG
 GDSNNITLFGESAGGASTHFLALSPQTEGLIHKAIVMSGSVLCPWTQPPRNNWAY
- 35 RLAQKLGYTGDNKDKAIFEFLRSMSGGEIVKATATVLSNDEKHHRILFAFGPVVE PYTTEHTVVAKQPHELMQNSWSHRIPMMFGGTSFEGLLFYPEVSRRPATLDEVGN CKNLLPSDLGLNLDPKLRENYGLQLKKAYFGDEPCNQANMMKFLELCSYREFW HPIYRAALNRVRQSSAPTYLYRFDHDSKLCNAIRIVLCGHQMRGVCHGDDLCYIF HSMLSHQSAPDSPEHKVITGMVDVWTSFAAHGDPNCESIKSLKFAPIENVTNFKC
- 40 LNIGDQFEVMALPELQKIEPVWNSFYAPNKL

>23:03:05|GENSCAN predicted CDS_1|1809_bp

atgggattcgatatggcaacacgctttatggatatactaaagctgacctttaagccatttaaaacgaactacactgaagaaaagtattt caatgacaaactcagatcttcgaaaaatattgaaaggcgttatatcttggatgttggctttcgcggacccacagcagtcacgtacaat ccaatctgggtaataagcttcaagtacgagcagcgcaaattgtcaacagcaatatattccgtcataaagacgaaatcaggtcctgtg cggggagtgaagagaaacacaatctggggaggaagctacttcagtttcgagaagatacccttcgcaaagcctccggtgggagat

165

ctgcgcttcaaggccccggaagcagtggagccatgggatcaggaattggattgcacttcgccggcagacaagccccttcagacaccgtgatggtttggatctacggaggaggctatcaagttggcgaagcttctcgaggattggatgtggtcatagtcaccgttgcttatcg actgggtgccttgggcttcctcagcctggatgatccccaactaaacgttcccggaaatgcaggtctcaaggatcaaatcatggccctgcgatgggtgcaacaaaacatcgaagcattcggcggtgattccaacaatattacactctttggcgaaagtgccggcggagcctc gaccacticcttgcactaagtccccaaactgaaggtcttatccacaaagctatcgttatgtcgggcagtgttttgtgcccctggacg caaccaccgagaaataattgggcttataggctggcccaaaaattgggatacaccggtgacaataaggacaaggcgatctttgagt ttctgcgatcaatgagtggcggggagattgtcaaggccaccgcaacagttctcagcaacgatgaaaagcatcatcggatccttttcgccttcggacctgtcgtagaaccatatactaccgagcacactgtggtcgctaaacaaccgcatgaactgatgcagaatagctgga gtcacaggatacccatgatgtttggaggcacgagcttcgagggattgctattctatccagaggtttcaaggcggccagcaaccctc gatgaggtgagtaactgcaagaatctgctaccgagcgatctcggtcttaacctagatcccaaactgcgtgagaactacggcttgca actgaagaaggcgtatttcggcgacgaaccctgtaaccaggcaaacatgatgaagtttctcgagctatgctcatatcgagagttctg gtgcaacgccattaggattgtactttgcggccatcagatgcgaggtgtttgtcatggtgacgatctgtgctatattttccacagcatgttgtog cat caat cog circ cog attot coggaa caca aggt tata accggaat t gtog cat cat get considered and the considered aggregation of the considered aggregationgateceaactgegaaagtataaaateacteaagtttgeaceeategaaaacgtaaceaactttaagtgteteaatattggggateagt

Drosophila Gene Hit TBLASTN with ORF1: alpha esterase (aE10) gene (U51054)
 Human Homologue TBLASTN with ORF1 and BLASTX with U51054: bile salt-dependent lipase (S79774)

Annotated *Drosophila* genome genomic segment AE003671
Annotated *Drosophila* genome Complete gene candidate CG1131 - alpha esterase 10

25
Human homologue of Complete gene candidate

4e-48 4557239 ref[NP_000656.1|pACHE| acetylcholinesterase (YT blood group) precursor >gi|113037|s

30

35

10

15

Putative function alpha esterase

Confirmation by RNAi

Only wild type profiles observed

166

CATEGORY 5: SMALL IMAGINAL DISCS (BLOCK TO PROLIFERATION)

Example 54 (Category 5)

Line ID 113/20

5 Category 2nd chromosome, small imaginal discs

Reversion R Map Position 50D/E

Rescue ID EcoR1

10 Rescue Sequence 1

CTGAGGCNCTTTGCCAATATGTGTATATTGGGCGGGGNACATGCGTNAATCGG
TTAAAGCCGCTACTTACATTCTGTTCTTTGCATCTCCCCCATCCACAGCTATAA
AGCAAGATGAGCTACGCCGCTGATGTGCTGAACTCGGCCCATTTGGGAGCTCC
ATGGTGGTGGCGACGCCGAGTTGCGTCCATTCGATCCCACGGNCCATGAT
TTGGATGCATCCTTCCGCCTTACACGCTTCGCANATCTAAAGGGGCGCGGCTG
CAAAGTGCCCGCAAGGAAGTNGCTCCCCCCACCT

Rescue ID BamH1

Rescue Sequence 2

40

20 CCACCTGTACCACAGCGCTCANACGTGTATGTACACGGATTTTCTGCCGCGT GTGTGTAGCGCGGCCCGTGATTGGCTGCAGTCGCGATGGCGGCTAAAACGGG CGAAGTCAGTATTTCTCCCTGTCGACGANGCGAGCAACGTGAACAATGCCCAC TCATTTCAATTGCAAAATGCCAAAAAGTGCGCGCTTTGAATTGGCCATTTGGT TCGTTGCGTTCGTTTGTCTTTTTGGTACTTACGTTTGCTGCGATTGTACAAA

25 GATAATTGTAGAGTAACGTTAGCAAATTATATTTATTTTGCGCCTGGTTTTTGC
TTTTCCAACGANCGAGATGTCACAACAGGGTTGTATTANCGTGTGCGGCTGAT
TCGATATTTGGGATGCCGATTGTCTGAAGCGANGGTTCAACGGGGCTGCCAAC
TCCCCCGAAAATCTATCNATGGTATTGTGCGCCAAGGGTAAAATAAATAAAAA
TATGTTAAAACCGCGGAATAAATGGGGGGAACCGAAGTGGAAACTGTGGTTCA

30 CAGTGCTCTGACTTTCGGGAGCAGTTAATATAGTTGGCATTAATTCAATTAGA GCTCCAAAGTGCTGGTCACAAAGAACGCACAAGAACGGGCCATGAAAAACCT GTTGCGCCAGCAGAACGAAAAGTAAAAATTAGAAGAAACCAT

Genomic hit, Accession No. CSC:AC017131

35 Drosophila Gene Hit rescue sequence: selenophosphate synthetase (ptuf1) (U91994)
Human Homologue BLASTX with U91994: SELENIDE, WATER DIKINASE 1

(SELENOPHOSPHATE SYNTHETASE 1) (SELENIUM

DONOR PROTEIN 1) (P49903)

Drosophila EST LD46437 (AI514756 similar by BLASTN to U91994

selenophosphate synthetase (ptuf1) gene)

167

Annotated Drosophila genome Complete gene candidate CG8553 selD selenophosphate

synthetase

Human homologue of Complete gene candidate 1711372 P49903

SELD_HUMAN SELENIDE,WATER

DIKINASE

(SELENOPHOSPHATE SYNTHETASE (1e-159)

10

5

Putative function selenophosphate synthetase

Confirmation by RNAi Only wild type profiles were observed

168

Example 55 (Category 5)

Line ID 121/1

5 Category 2nd chromosome, small imaginal discs

Reversion NR Map Position 60B

Rescue ID BamH1

10 Rescue Sequence

35

TCCTGTGCACTCATATTGATTTGCCTTGTCAAGTGGCTAAAGAAATATTAAATG
TTTGTTATTTCTGTTGCTAGCGCTCCGACAGTCTGGCAGCACTGCTCGCTGTCG
ATAGTTCAACTGAGTTGCTGTTTCATCGAACAGAGCTGCCAACTCTATTTTTGT
AGCTGGCCAGCCAGGATTGCCAGAGTAAGGCCCTCAAATCAGCTGTTTTGTGT

- 15 TTTGATTTTATTTTGAAAGTCCTAGTTTAAAATTATGCTTTCTCCGACAGATCA GCACAAATAATACTAATAAAGCTCACAATGCTAAGGTTGTGCCTTCCAACTCG AGCTGGATATGTGCGTAAGTAAGGACTTTACGTCTATAAAACTTGTTATGTAA AGTAAATGTTTGCCTATTGCGCAATTTCTCCAACGAAAAACCCAGAAAACCNA AACCCCCTTNAAANTTTGGAATATNCCCAATGAATGCAGCACCCGTGAAATCC
- 25 Genomic hit, Accession No. CSC:AC020499

Drosophila Gene Hit rescue sequence: DnaJ60 gene for dnaJ-like protein (Y11900)

Annotated *Drosophila* genome genomic segment AE003463
Annotated *Drosophila* genome Complete gene candidate CG12240 – DnaJ60

30 CG13570 – spaghetti ser/thr

phosphatase

Human homologue of Complete gene candidate CG12240- 4827026

ref|NP_005138.1|pTID1| tumorous imaginal discs (Drosophila) homolog >gi|3372677 (AF061749) 7e-

80

40 CG1116- 2495728

HYPOTHETICAL PROTEIN

KIAA0258(aa)

45 Putative function CG12240: Chaperone involved in protein folding

CG13570: serine/threonine phosphatase

PCT/GB01/01297 WO 01/72774

169

CG12240: Marked reduction in G1 and G2/M peaks indicating fewer cycling cells CG13570: Marked increase in G1 peak Confirmation by RNAi

5

170

Example 56 (Category 5)

Line ID 127/2

Category 2nd chromosome, small imaginal discs

5 Reversion NR Map Position 57F

Rescue ID EcoR1

Rescue Sequence 1

25 Rescue ID BamH1

Rescue Sequence 2

CA

CTTCTTTTCTCAAAAAACGTCGCTCGNGTCCCNCAATCGTTTTACAAACTTCGC
TCGGAACGGACGTGTGCGCGCTCTGAAAGGAAAAAGTGAAAAAGTGTGTGAC
AAAGTGCAAATAAGCCACAACGCGCATGTGAGAAATCAAATTTAATTGAGAA
GCATCAAAAATTGTATACATATCGAGCGTATCCACATCGCTGTATGTGTGAGT
GTGCCAGTGCTAGTGTGGTTTTCCCTTTTCGCCGTGGAAAATATGAAAACTGA
ATGAAAAACTGAATCGCAGTCAGCCAGAGCCGAATTGGAAAAAGAGTAACTCG
CATTGGGGACACGAAGAGGTGTCTCGAAAAAAGGTAAAATCTTTTACACAGAA
ACGACGCCAGAAAGCGATTAGCGATTTNTGACTATGTGTGAGTGTAATTTC
GGTCTACGGCTGTGTGTCTCGCATTTTATTTAACNTTTTGTTTCCCNGTTNGNTC

35 GGTCTACGGCTGTGTGTCTGCATTTTATTTAACNTTTTGTTTCCCNGTTNGNTC CACNGTAAAAATAGCTAAAAAAAAAGGGCAAGTACTCTTGGCGCGCTCTCCC TCTCTCTTTGTTGGTCGTGACTGCGACGTCACCGTTCACGTAGAATCGTTTTCA AGTGGCGTTTCTTTCTTTTTTAATGTGCTGCTTCTTGCTTCTGCCTCTTCTTC TTGCCTTTGGCTATCTGCTTTTTTGAAATACGTCCATGTTATTCCAGTGTCTG

40 TGCCAAATGTGTGCGANATGATCTCTACTT

Genomic hit, Accession No. AC009732

Associated ORF

45 Genscan ORF1 predicted sequence
>/tmp/aaaaafrla|GENSCAN predicted peptide 2|456 aa

MOTKGPITDADCIRGMACRALAGLARSDRVRQIVSKLPLFASGQLQTLMRDPILQ EKRAEHVIFQKYALELLERVSGKTKPLNNPLDPSLSNMHKANVIAQTRIQYNKQQ LYOLIFEHLESNGLSQTAQMLQREVGLPLQTPTTRSFHQSPFDYKSLPSGSSSLSRN RLRSRMQDVNAAIMGNGDLNRSFGEDSSPAGAGGSNAGDGVSIPNFSSLNTTQTP IKIRRTDRSSVSRSIOKOAMEPGGMSVGLAEDGQLHPKRITLNTIVTEYLTNQHSL CNNPVTTCPQFDLYEPHKCPDPKPSRLLSSNYNLTSRHARTQAGFNTSRFDRRYV HTHFSPWRSIRSADYEDLEFTCCDLAGKYIIVGTQQGDGRVFNMNDGVEQFFSNC HNFSVDAIKANRAGDLVITSSFWRTPTSILWSIADDEFKLKLRLPDVTYCEFSQTV *QDRLLGTQNEVY*

10

15

20

25

>/tmp/aaaaafrla|GENSCAN predicted CDS_2|1371_bp gggtcaggcagatcgtcagcaagcttccactctttgccagcggacaactccagacgctgatgcgggatcccatactccaggaga agegegeggaacatgtaatettecaaaagtaegeattggagttgetagaaegagtgtegggtaagaegaaacegetaaataatee tttggatccatcgctgtccaacatgcacaaggccaatgtaatcgcccagacacgcatccagtataacaagcagcagctgtatcagc ttatcttcgagcacetggaaagcaacggtctctcccagacagcccaaatgctgcaacgggaggtgggtcttccgctacagactcc ggtggtagcaatgcgggagatggagtcagcataccaaattttagctcccttaacacacgcagacgcccataaaaaataaggagg acggatagaagttcagttagccgctctatccagaagcaggcaatggagcctggtggcatgtcagttggtcttgccgaagatggtca $actg cate cea agagg at cacceta a ataccate \\ \dot{g} ta acc ga at acctea cea accage acteg etg t gea at a at ceg g t ga cacceta \\ \dot{g} ta acceta accage acteg etg t gea at a cet caccage acteg etg t gea acteg etg t gea$ acctgcccgcagtttgatttgtacgagccgcacaagtgtccagatccgaagcccagccgattgctaagctcgaactacaacctgagcattcgatcggcggactacgaggacctagagttcacctgttgcgatttggcgggtaaatacatcattgtgggcacgcagcagggcgacggacgagtgttcaacatgaacgatggcgtggagcagttcttctccaactgtcacaactttagcgttgatgctattaaggctaatagageeggagacttggteateaeatetagettetggegeaeaeceaecageattetatggtetattgeggaegatgagtteaageta aagttgcgacttcccgatgtcacgtactgtgagttcagtcaaacggtgcaggatcgtttgttgggcacccagaatgaggtatactaa

corresponds to CG10082

30

several including SD04293 (AI532704) Drosophila EST

Annotated Drosophila genome genomic segment AE003454

35

Annotated Drosophila genome Complete gene candidate CG10082 - novel protein with homology to enhancer Pi uptake

Human homologue of Complete gene candidate

1665793 dbi|BAA13393| (D87452) Similar to S.cerevisiae YD9335.03c protein (S54640) [Homo sapiens] (2e-43)

40

Putative phosphatase or enhancer of Pi uptake protein 45 **Putative function**

Reduced G1 and G2/M peaks indicating fewer cycling cells Confirmation by RNAi

Example 57 (Category 5)

Line ID

131/8

Category

2nd chromosome, small imaginal discs

Reversion

R

Map Position

60A

Rescue ID

BamH1

Rescue Sequence 1

- 10 CACGATTGCNGGCCCATCGAAGTGTGGGTCTATCGATACTCGTGGGTAAATAA ACAAGTTCTGAACTGCGATTTCGGGGGTTTGAGGGGTCAATTGTCCCCTGTGT TGGAATGTGTTCCTAAATCTACACAAACACTCCCTAAGCTTATCCTAAACTTAT AAATATTGGTTGCTATTTAAACCCCATTTCACGGTTATCCAGCACGCCCCTGA ACTGTGACCCACATCCCCGATTTTAGTGACTAGTTTTATACTTATCGTGGTTGG
- 15 CATTTGGTACACTACACTTTCTTATTCACCTAGATCGCCGACTCCGCGCACGGT CGCGCTCCCGTTCCCGATCTCGGCTGCGACTGCGGTCGCGATCCCGTT CCCGGTCGCGGCGACCGGCGCCTCCANATCCGGATCCCTAANCGGCANCNGT CNTGGTGGCAATCNNGGAATGTTCCGGGGNNCCNCTACCNCAGTGNAATCAC TGGTACGTCCCACCGCNAAACTCCGCCCANTGCGGTTGCCGGAACGGGTGGC
- 20 ANTGCCAATGGGTCGCTGCAGAAGGTACCATCACAGCAATCGCTCACGGANC CCGAAGACTGCCTCTGCCGCCCGGCTGGGCCACTCATACACGCTACACGGTCG GAAATACTACATTGATCACAATGCGCATACCACGCACTGGAATCATCCGTTGG GAACGC

25 Rescue ID

EcoR1

Rescue Sequence 2

AATTGATTTCCGGACATATAAACAGAATCCAGAACTCATCCGGCAGCAGGCTC AGTCAGGCCAGTAAATCCGAAAAGAGAGTAACCAGCAGGAAAAGAGAATCC ACGTAAATACAGAGAAAATGGCTCTACGCGTCCAATTCGAGAACAACGACGA

- OATCGGCGTATTCACTAAACTAACCAACACATACTGCCTGGTGGCCATCGGTGGATCCGAGACCTTCTACAGCGCCTTCGAGGCGGAGCTGGGCGACACCATCCCGGTGGTGCATGCGAATGTGGGCGGCTGCCGGATCATCGGCCGCCTCACCGTGGGCAACCGCAACGGCCTGCTGGTGCCCAACTCCACCACCGACGAAGAGCTGCAACCCTGCGTTACANCCTGCCANAACCCCGGAAANATTTATCGTGTGGAAGAAC
- 35 GCCTGTCCGCGCTGGGCAACGTTATCGCCTGCAATGATTATGTGGCCCTGGTG CACCCGGATCTGGACAAGGAGACCGAGGAGATCATCGCGGACGTGCTCAAAG TANANGTCTTCCGCCAGACCATTGCCGACAACTCACTGGTGGGCTCTTACGCC GTGCTGAGCAACCAGGGGGGCATGGTGCATCCCAAGACNAGCATTCAGGAAC AGGACAACTGTCGTCCCTGCTGCAGGTTCC

40

Genomic hit, Accession No. CSC:AC020517

Associated ORF

Genscan ORF1 predicted sequences >22:13:05|GENSCAN_predicted_peptide_4|357_aa

MALRVQFENNDDIGVFTKLTNTYCLVAIGGSETFYSAFEAELGDTIPVVHANVGG
CRIIGRLTVGNRNGLLVPNSTTDEELQHLRNSLPDAVKIYRVEERLSALGNVIACN

DYVALVHPDLDKETEEIIADVLKVEVFRQTIADNSLVGSYAVLSNQGGMVHPKTS IQDQDELSSLLQVPLVAGTVNRGSEVLAAGMVVNDWLSFVGMNTTATEISVIESV FKLNQAQPATVTTKLRAALIEDISRSRVAGGGGGGGGGGSSGGNSSSGPSTSRRTT RNNAAATAADRPKINEADLEGKSPEEVEMLKTMGFCTFDTTKNRKVEGNDVGEV HVILKRKYROYMNRKGGFNRPLDFVA

>22:13:05|GENSCAN predicted CDS 4|1074 bp

atggctctacgcgtccaattcgagaacaacgacgacatcggcgtcttcactaaactaaccaacacatactgcctggtggccatcgg tggatccgagaccttctacagcgccttcgaggcggagctgggcgacaccatcccggtggtgcatgcgaatgtgggcggctgcc ggatcatcggccgcctcaccgtgggcaaccgcaacggcctgctggtgcccaactccaccaccgacgaggagctgcaacacct gcgtaacagcctgccagacgccgtgaagatttatcgtgtggaggagcgcctgtccgcgctgggcaacgttatcgcctgcaatgat tatgtggccctggtgcacccggatctggacaaggagaccgaggagatcatcgcggacgtgctcaaagtagaggtcttccgccag accattgccgacaactcactggtgggctcttacgccgtgctgagcaaccagggcggcatggtgcatcccaagacgagcattcaggaccaggacgaactgtcgtccctgctgcaggttcccctcgtggccggaacagtgaaccggggcagcgaagtactcgccgccg gcatggtcgtcaacgactggctctccttcgtgggcatgaacaccacggccacagagatctccgtgatcgagagcgtcttcaagcttaggaacaatgcggcggccacagctgccgaccggcccaagatcaacgaggcggacctggagggtaaatcgccggaagaggt cgagatgctgaagacaatgggattctgcacgttcgacaccaccaagaacaggaaggtcgagggcaacgatgtcggagaagtgc at gta at cetea age gaa ag tace ge cag tacat gaa te ge cag get ge cete gat the general gas to get the general gas and general gas the general gas and genera

Drosophila Gene Hit rescue sequence and TBLASTN with ORF1: b(2)gcn

(EUKARYOTIC TRANSLATION INITIATION FACTOR 6

)((X97641)

BLASTX with X97641: integrin beta 4 binding protein (HUMAN Human Homologue 25

EUKARYOTIC TRANSLATION INITIATION FACTOR 6)

(NP 002203.1)

GH08760 (AI109537 similar by BLASTN to X97641 Drosophila EST

"D.melanogaster b(2)gcn gene.")

30

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AE003462 Annotated Drosophila genome genomic segment

CG17611 - bcgn benign Annotated Drosophila genome Complete gene candidate

gonadal neoplasia homology

to Eif6 translation factor

35 Human homologue of Complete gene candidate

6016331 EUKARYOTIC

TRANSLATION

INITIATION FACTOR 6 (EIF-6)(aa) and 4504771 |ref|NP_002203.1|pITGB4BP|

integrin beta 4 binding

protein(aa)

eukaryotic translation initiation factor 6 (eif-6)(aa) Putative function 45

Slightly reduced G1 and increased G2/M indicating block in Confirmation by RNAi G2/M

PCT/GB01/01297 WO 01/72774

174

Example 58 (Category 5)

135/25 Line ID

2nd chromosome, small imaginal discs Category

Reversion NR 24A Map Position

> EcoR1 Rescue ID

Rescue Sequence

ATAACATGGGCNCTGGTTTTTAAGTNAAGCTCTANTNATTGGCCCCCATTCTTA 10 NNCTCTCTCGCTCTTCTCGCCTCTTTCGCCTGCTCTCTCGCCTGATTATTCTGC TTGGTCGGCTGATGGTTTTTNGTTTTNATCTGGTGTATTTTCTGCGTAGTTTATG ACAAACCGGCTGGTTCTTGTTGTTATTGCCGTATTCTAATATATTCCCCTATTG TTCTTATTTTTGTTGCAGCCTGCACACCTCGGAGGTTCTAGATGATAAGGGGTG TAGCGATGGTGGGGGGCTGTCTTGANGGGCTTCTCGCCTTGAGCTCTTGTTTAT 15 CTTTGGTCATTTGTTATTGTTTAATGCACGGCAATATTATTGGTAAACAAGTTA GCCAACAGCACTAAACGCCAATCGCATTCTTTTCTAAAAACCAAGTCTATTGT CGATCTTGCTAGGGAAATGATGATGACTCAGGTGCAATTGGGATCTTATCTAT GGCTGTCTGGGAATCAAGAAGTGTTCCCGCAGAATTCGTGAANTACTGCCGCT CTCTCCATGGGGCCATTATTTGCACTCGTTTTNCGCGAAATACCATNAATTAGC 20 ATAAAGACACGTCGCCGGCAATCGTGACGTAGGCTATNAATGCCTTCTATGCA TGTGCNAACTCGCGGAAGCATAGCAATTTGAAGGAACAATATTTCANTGCAG **GTTTTAATGGGCTAAAAAA**

Genomic hit, Accession No. CSC:AC014199 25

Associated ORF

Genscan ORF1 predicted sequences >20:54:54|GENSCAN_predicted_peptide_3|117_aa MSASPTARQAITQVMPMITRKVVISDPIQMPEVYSSTPGGTLYSTTPGGTKLIYER AFMKNLRGSPLSQTPPSNVPSCLLRGTPRTPFRKCVPVPTELIKQTKSLKIEDQEQF 30 QLDL

>20:54:54|GENSCAN predicted CDS_3|354 bp

at gtccgcttcacccaccgcccgtcaagccatcacccaggttatgcccatgatcaccaggaaggttgtcatctcggatccgatccagatgcccgaggtgtactcctcgacgccggcggaaccctctactccaccactcctggaggcaccaaacttatctacgagcgggc 35 aactggatctgtag

Drosophila Gene Hit TBLASTN with ORF1: BcDNA.HL08053 mRNA (AF132557) TBLASTN with ORF1 and BLASTX with AF132557: eukaryotic Human Homologue

translation initiation factor 4E binding protein 2 (EIF4EBP2)

(L36056)

45

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175

Annotated Drosophila genome Complete gene candidate CG8846 - phas 1 translation

initiation factor 4E binding

protein 2

Human homologue of Complete gene candidate

CG8846 - 4758260

ref|NP_004087.1|pEIF4EBP2|

eukaryotic translation initiation factor 4E binding

protein 2 (4e-16)

10

5

Putative function EIF4E translation factor binding protein

Confirmation by RNAi Slight reduction in G1 and G2/M indicating fewer cycling

15 cells

Example 59 (Category 5)

Line ID 141/12

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 21A/B

Rescue ID BamH1

Rescue Sequence

10 GGCTCTTTTCCAAANAGGCAGTTTCTTGNCCCATTTCTTGGAITGCTTTGTAGT
GAACTNAATCGTTTTGTTGGTTCCTCTGTCGTCCAGTCTTGTGAAAAATTTCGTG
ATAATAATGCCTGGATAAATANTTAAGCATTTGGAAAACGGGGGAAAAAGGG
CTAAGTTGTGTGAAGGAAACAATTGAAGTGACCCTTTGTNTATAAACATTCCA
CGACGTGTTTCGAAAACAAACAAAGATATGCGGAAACAAAGTGTTAATAAAA
15 GAGCNAAAAATAGAGAGAGAGTGTCGCGATAAGCGGTTGAGCGAGATAGAG
AAAATTGTTGATTAAAATGTGTGTCNAAATAAACATCAAGCCGCTTGAACGA
ACAGTCAGTTAGTTGCTTCTGATAATAACCATGGGAAGCGGCNCGTGTGCTTC
GCTCCTCGTTACTTATAAAATATTTAAACGTTTGCATTCTTCNTATTTCCGAAT
TTTTGCNCCCCTGAANCAACTTNGTTAAACTGCAAATAGCAATGCAAACAAAC

25 Genomic hit, Accession No. CSC:AC017815

Associated ORF

Genscan ORF1 Predicted sequences >17:48:30|GENSCAN_predicted_peptide_2|554_aa MSNKKMFNRTTSVSPGQLHYYHTDFYYSMPDLHKTRKMHGVKRVLVFCLMIVIL

- PAILIIMPLHLRKTVFADVIYPMAESDIIEIRAGISSIFCSKHTLRMNSNFNAFQLRNK PEIATNRKHIRLKKSMTLPDDTLEYWGFFLLKGAKVRVKFCSRYDGSRILIIHGHR ELNLCGLTDHNKNKLGANYAKGHEQVQVFFEDNVEITEEKGNQDVLMEHENHG GEDLTEDIPQPQVNIPVKQNNSIQPKLIRKKLKKGTIHHGEHDMHAITDLQGSHHT EHILNHDHSSNSPAHHHNSTAHHREHSSNITNEETSRNHIRNEDEDPDQNSSKTH
- 35 YSAESPPHRERLKRHNRVAHRNQKRQDLYDTLYKRSKRENVYDRKTIHGGNAIN FTETDESNSVSSFETGLFQCFNGMILLQEFFRPKNECSNPHIMDTSPNKSSMVVHN VIEDGYYYYIFYSDNDHVQNEIHAIFDIYKPTYQYSNMSESQSCLNTTNCTFNISFL SDEIVVVEVPTRDGIEHEEDDITNLISTCHPRSEIYAIFPITVLVLILCCSFL
- 40 >17:48:30|GENSCAN predicted CDS 2|1665 bp

45 gaatcgcaagcacattaggctgaagaagtcgatgacattgccggatgatacgcttgaatactggggcttcttcttgctgaaaggtgccaaggtgcgagtgaaattctgctccgctacgatggatcccgcatcctgatcatccatggtcacagggagcttaatctttgcggtct

177

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10

corresponds to CG9524

Annotated *Drosophila* genome genomic segment AE003623 Annotated *Drosophila* genome Complete gene candidate CG9524 - novel His-rich protein

Human homologue of Complete gene candidate

Putative function No homologies which indicate function

25

Confirmation by RNAi Reduced G1 and G2/M peaks indicating fewer cycling cells

none

Example 60 (Category 5)

Line ID

146/2

Category

2nd chromosome, small imaginal discs

Reversion

NR

Map Position

26B

Rescue ID

EcoR1

Rescue Sequence

TTTNATCCAAACTGAGANACTNTTGGCCCCAAAACTGAAAACTCGGACTCGGG 10 CGCGTAAGGGAGTCGGTCNTCGGGAGTCGGTCGTCTTTTGTTGATCTTGAGAC TGAAATTCCAATTGTTGATTTATCTCTCGGCTGCTGCGCCGCGGCTGCGCTGCT GCAGCGCAGTCCCACTCCGATTTGACCAGCGACCAAGTTTATAAAACTTTGAG CCAAAATGCAGCGGCGCACAGTTGTTACCAAAACGTTGCACGCGTCGTGGCCC TCATCAAAACAAAAAAAAAATATAAGCGAAAATGAAAACGAAATTCGGTTA 15 ACGTCAACAGAAGCTGACAAAAGGCAGAAAAGACCGAAACAAGTTGCAGGG CCAGAGTAAGCCAAGTTAAATGCGAAAGAGAAGCAAGAGNCAAGAAAAN AATGGGCACTACATACATATTATAGCCAGCTAATCTGTTGTGCAGTGCGTT TTATCAGCCNNCGAAAAGAAAACGAAAACGAAAAGTCGGTCCAAGTTCGGAC TCAAAATCCAAACAGAAGAGACTCCATNCCATCAGAGACACGCGGATCTCAT 20 CTCGGTAATGTCTCAATAAAAGTAATCTTAACTGCCGCCGGGAATGTTGGAAA AAGTGAAAATTGAAGCGCTTAACGTGTTTCGAAATACGATACATGAGAAGTCC CAAAAAAAAAA

Genomic hit, Accession No. CSC:AC019865 25 GH19286 (AI388389) Drosophila EST

Annotated Drosophila genome genomic segment

AE003481

Annotated Drosophila genome Complete gene candidate CG11353 - novel with weak

homology to sugar acetylase? CG7525 - tie receptor protein

tyrosine kinase.

Human homologue of Complete gene candidate CG7525- 4e-23 4557869

ref|NP 000450.1|pTEK| TEK tyrosine

kinase, endothelial

>gi|464868|sp|Q02763|TIE2_

Sugar acetylase and receptor tyrosine kinase Putative function

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Confirmation by RNAi

Both gave a reduction in G1 and increase in G2/M peaks

indicating arrest in G2/M

PCT/GB01/01297 WO 01/72774

179

Example 61 (Category 5)

Line ID

155/13

Category

2nd chromosome, small imaginal discs

5 Reversion R

Map Position

21B

Rescue ID

BamH1

Rescue Sequence 1

10 GNTTTAGTCCNCTTTTGANAGGGNCTTGGNGNCTTAAANAANNAAAAAAGGG GNCCCGGCNCCCAGCAAANAGNNTAAAACTTGAATGGTTTAATTCGAAAATC TTTTAGAAATGTCGCCTAATACCTTATCGGTATAGAGTTCACCTCGTCTCCTAA TCCATATTTTAAGATATCAATATCTATTAACAATTTTTATCGTATGATTAGAAA TTCGCATTGTTTTATTATTTCGACCTTTGGGCTTTACATCGACAGCTACTCTCTA TCCAGACAGGAGACTGGGAGAGAGAGCACGATGCTGTCTGAAAGCATGAATG ATGGATGCTGTGCCTATGTGCGATATGCACGTTGCCTGAGCTAAAACGAAACG GCTTCAATCGCNCTNTCGATNTGCGACAGNGACATNTTTTTATCTTCGACNAT 20 GCNCTCNCTCCCTCCCACAGAAATCTTGCGCTNGNTCTCCGANNTNGGGNTNG ANGGCNCTCTTCTCTNTCCTTAAATTGGGANTTNNCTTTTTTCNAANAAGGGN **NAGA**

Rescue ID EcoR1

25 Rescue Sequence 2

AATCNTTTTNTCCATTNGGCGNCTTNCTCAAAACATATTCACATTTGGNCCCAA CGGCGTANGACTTNATCTCACGATTGTTTGGTTTCCTACTCTCCCGCGCTCCCT CTCTTCTGAGTCTCTTTCTGGCTGATTCGCATTCGATTTTAGCCGCTGCCATCG CCGTTGTTTTGCCTACCTATGTGTGTGTGTGAGGAGTGTGTCTTGTATTTCAGT CCGCAATGCGCTCCGCTCATTATTTGTTTGANCGCCGCGGTGTAAAGTTGTAA AAAGTCCAAGTGCTCGTGGAAACTCGATGCAAGACGGGGAAAACGAAACGCG ATAAATCGTGAGAAAAGAGAGTGCGCTAAAGGAAGAGGGAGTGATAATCAN ACGAAATGGAATAATGTNTTTGCAGAGGCNACAACAACAATGCAAATAGTTG TCATTGAGGCGCAATGAATGATAATTAGTGCTTANTTGAAATCATAATCNTGA AGAAAGCGTAAAGCTCGATTNTGGCAATNTATTCTTGATTACCANTGAGTCTG TGATATTGCCGTGTGTNCCGAAAATGGANGTTATNAAACCCATGGACTTCAGC ACCTTCTCCGCGTTCTGCGAACATCTTAACAAATCTCCACAAAATTGCAGCAA

CAACTGCANCGACGGTACCGCCAACTATAANCAATGGAAAANGCATTATTTG GAGGTAANAGCNAAAAATACCAATNTTTCCAATGCGAAATTGCNAGCNTGG

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Genomic hit, Accession No. AC004274

Annotated Drosophila genome genomic segment AE003590 Annotated Drosophila genome Complete gene candidate CG13693 - novel

45

Human homologue of Complete gene candidate

6e-05 4507659 translocated

180

promoter region (to activated MET oncogene)
>gi|1730009|sp|P12270|TPR_HUMAN POOR MATCH

5

Putative function No homologies to indicate function

Confirmation by RNAi Only wild type profiles observed

181

Example 62 (Category 5)

Line ID 162/24

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 55C

Rescue ID EcoR1

Rescue Sequence 1

TTTTNTTCANGGNTCTTTGCNCATAAAANACACGNGCCCTCNTGTCCATTCAC
ATTTTACTTGGAGTCGGTAACGTTGAGTTCCGCGTCCGTGCGTTCTGCCTTCCA
ATACAAAGTCTGGTGGAATCTACCAAGCATTCCAGTGNGAAAATCAACTCAC
ATTGCTCGGTGATCCNTGCGGCGGTATNATCGCACCCGGAATTGCATAAGTTG
CGGNGAGCGGAAAGAGAGTGCACGGATTTNCNGTTATCNAAGGGCCGGCANC
NGTGGGGCGGCGACGGNAGAGCACGCAGAANAANAATANANTGNNGTGGCG
AATTNAAAAATANNATNAAAGAAAATTCGGGCCGCTAATTTTCTTCAAATTT
GTGTGCGGTCGGCGAAAAACAACGTGTTTTTCNATGGTTGATAATACACACGG
ACGGNNCACTCGCGCTCACCCACATAGTCACNAAAGTCGGCGACGTCGACGA
CCCNCACNCTCACATANGGACNTTTAATCCCGTNCATNCGTGTAGCGTNCNTA
TTTAACCNTNTCTGTCCATCGGAACGCNCGCNTTCTCGCCTTCNTTCTNTCCTNT

TTTAACCNTNTCTGTCCATCGGAACGCNCGCNTTCTCGCCTTCNTTCTNCTTTA
CTTTAATTTCCTATTTNNAAGGGGNAGNCCNATCTTTTTNCCTNTCNNTGCCNT
TTAANNTCATCCACANCCTCNCTTTNTCNTTCCTCCNCCTTNTNTTCTTTCNTC
TTNCTTNTGNCCTTGCCTCGTTCTTTCTCTTCNTCTCCTTNCCCTTCTCCTCCTTT
TTTCTCCTTCCCCCC

25

Rescue ID BamH1

Rescue Sequence 2

AAGNCNCCTTGGCCGNNTTNAACGGNAANTAANCCGGGNCCNCGGGNCNCGA TAATCAGGTCNANCCTTGTGCCTACCACCACCAAATTGAAAAAGAGCNAAGA 30 TTCTCTAAGGCAAAAACTCCCCAATCTGTGGAATTTCCGGAAGCGAGAGCAC ATTCAAAGCTACCAGTTATCAGCGAGCAGCATGTCTAAGCTCAGGAACCTGTT GCCCACAATCTTTGGCGGGAAGGAGGCACAGAATCCGACACCCGTCGAGGGA CGCCTGGAATAGGACGCAGCTCCCGTGGACGACAACGAACCNGATTACTACT ACTGCGGAGCCATGGCGCTGCCCTCCACCGCTGGCACGCCCACAGCCTCCTCG GATCTGACCGAATCCGTGCTGCGCGAGCTCAGCGACCCAAACTACAATTCAAT 35 GGATGTGGTGCTTTCNNCCTNTTTTCCGGGCACTCTCAGTAACGTCCAGACAA ACAACACCATGAACGTTCACNGCGCCCAGCAACAGGTGGTCATGAACTTCTCG AATGCCAATAATCTGCACTTCGGCTCCGTCTTCAACTTCAACCAAAACTTGAG CGCCTGCNGCTCNCGAANGGGTTTCACCNGTTCGCANAAGAATCGGTCGCCTC **TCCANACNGT** 40

Genomic hit, Accession No. CSC:AC012981

Associated ORF

45 Genscan ORFs: ORF2 predicted sequences >18:26:17|GENSCAN predicted_peptide_7|1320_aa

MEETNNATTIEQQPIALINGQEQVANEQQPSSPTSVATPTSTTSGGTGNATPAFSY DDLFPALPANTSAQSQSGASGSTLARVTSSQKTHIVHVPCKERKSTESEKFGEGES KRICQQITKETGAQIEIASRQVTVPREHFRVILGKGGQRLREIERVTATRINIPSQSD ESEFITIAGTKEGIAQAEQEIRQLSAEQYKKSSDRITVPKVYHPFIVGPYSENLNKLQ EETGARINVPPQQVQKDEIVISGEKDAVAAAKAKVEAIYKDMEKKCSTVSVEVAK PKHRYVIGPKGSTIAEILQLTGVSVEMPPNDSPSETITLRGPQVALGNALTVVYQK SNSVKSVEINAAHWIHKYVFGRKGANMKQLEEDCPNVNVNCLEDKIKLEGDPEN VDRAVAYLSEIIKNYEENFTFEVMTVNPSYYKHIIGKAGANVNRLKDELKVNINIE EREGONNIRIEGPKEGVRQAQLELQEKIDKLENEKSKDVIIDRRLHRSIIGAKGEKI REVKDRYRQVTITIPTPQENTDIVKLRGPKEDVDKCHKDLLKLVKEIQESSHIIEVPI 10 FKQFHKFVIGKGGANIKKIRDETQTKIDLPAEGDTNEVIVITGKKENVLEAKERIQK IQNELSDIVTEEVQIPPKYYNSIIGTGGKLISSIMEECGGVSIKFPNSDSKSDKVTIRG PKDDVEKAKVQLLELANERQLASFTAEVRAKQQHHKFLIGKNGASIRKIRDATGA RIIFPSNEDTDKEVITIIGKEESVKKAREQLEAIIKECDEVTEGEVSVDPKHHKHFVA KRGFILHRISEECGGVMISFPRVGINSDKVTIKGAKDCIEAARQRIEEIVADLEAQTT 15 IEVVIPQRHHRTIMGARGFKVQQVTFEFDVQIKFPDRDATEPVEGLTNGGSGENG GENEGQEGEQEVEKEAEQEPVRQCDVIRITGRIEKCEAAKQALLDLIPIEEELSVPF DLHRTIIGPRGANVRQFMSKHDVHVELPPSELKSDVIKVCGTPARVAEAREALVK MIEDYEADRADRELRSFVLQVDVDTEFHSKLIGRHGAVINKLRADHDVIISLPKRD EPNDRIISITGYQANAEAARDAILEIVGDPETLHREVIEIDKRIHPHLIGQRRRTIRKII 20 EDNKVNIKFSADDDNPNSIFISGKIEDVENVKELLFGMAEDYERDYLDNVAIAPPTI GAFLTGFWIRCRRCQRERIRHQRRTVGEAKAGQKPDCAQHSVAGGLPALRCWRG SGGLHAYHLRVGPQKLSASGRVSRSPAVAAILQVGVRRGSELEMDQELEQKLELE LELDYRAMSGRAAAVVRTSL

25

>18:26:17|GENSCAN_predicted_CDS_7|3963_bp atggaggaaactaacaacgcaactaccatcgagcagcagcccatcgctctcattaatggccaaggaggaggccaacgagca gcaaccatcctcgccaacttcagtggccacgcccactagtaccactagcggcggaactggcaatgccacacccgcctttagctac gacgacct gtttccggccctgccggccaacacttcggctcaatcgcaatccggagcttccggttcgactctagctcgtgtgacgagttcccaaaaaaactcatattgtgcatgttccctgcaaggagcgcaagtccacggagtcggagaagtttggcgaaggcgagtcgaagtcgaagtcgaagtcgaagtttggcgaaggcgaagtcgaagtcgaagtcgaagtcgaagtcgaagtttggcgaaggcgaagtcgaag30 cgt att t t g c ag caga t cac ca ag g ag ac c g g ag c c ag at t g ac ag t t g c ag t t g ac ag t t g c ag t g c ag t t g c ag t gcgatgagagcgagtttatcacgattgccggaaccaaggagggtattgcccaggccgagcaggagatccgtcagctgtcagccgagcagtacaagaagtcatcggaccgcatcacggtgcccaaagtttaccatcccttcatcgtgggcccctacagcgagaacctaaa 35 aaggacgcggtcgcagcggcaaaggccaaggtggaggccatttacaaggatatggaaaaggaagtgctctaccgtcagtgtgga ggtagctaagcccaagcaccgatatgtcattggtccgaagggctccaccatcgccgagattctgcagttgaccggtgtgtctgtag agatgcctcccaatgactcccctcggagacgatcactttgcgtgggccgcaagtggctttgggaaatgccctaaccgttgtctac caaaagtccaactcggtcaagtctgtggagatcaatgcggcacattggatccacaagtatgtgttcggtcgcaagggggccaaca 40 cgttgacagggctgtagcctacttgtccgaaatcatcaaaaactacgaggagaacttcacattcgaggtgatgacggttaatccttc gtactacaagcacatcatcggtaaggctggagccaacgtaaatcgcctgaaggatgaactgaaggttaacattaacatcgaagag acaaactggaaaacgaaaaatcgaaggatgtgatcatcgaccgccgtctccatcgttctattatcggagctaagggcgagaagatt cgcgaggtgaaggaccgctaccgccaggttacaatcacgatacctacgccccaggagaataccgatattgtgaagctgcgcgg 45 cccatcttta ag cagttccaca ag ttcgttattggca ag gg cg ctaacatca aa aa ag at ccg cg at gag accca gactaa aa tag ag accca gactaa acca gactaa

tecannagattenanaegagettteegacattgteneegaggggggeanateeegeecangtaetaenaeteaateateggeaet ggcggcaaactcatctcctcgatcatggaggaatgcggtggtgtttclatcaagttccccaacagcgactccaagagcgataaggt accgccgaggtgcgccaagcagcaacaccacaagttcctgatcggcaagaatggcgcttctatccgtaagattcgcgatgcc actggtgcccgcattatcttcccttcaaacgaggacactgacaaggaagtgatcaccatcattggcaaggaagaaagcgtaaaga aggcccgtgagcagctggaggcgatcatcaaggagtgcgacgaagtaaccgaaggtgaggtttctgtcgatcccaagcaccacaagcacttcgtggccaagcgtggcttcatcctgcaccgcatttcggaggagtgcggcgtgatgatctccttcccccgtgtcgg catcaactccgataaggtgacgatcaagggtgccaaggactgcattgaagcggcccgccagcgcatcgaggagatcgtcgccg atctggaagcgcagaccaccatcgaggtggttgattccacagcgtcatcatcgcaccatcatgggcgcacgtggatttaaggttca 10 gtcagtgcgatgttatccgaatcacgggcagaattgagaagtgcgaggccgccaaacaggctctgcttgatcttatccccatcgag gaggagttgtcggtgcctttcgacctccatcgtaccatcatcggaccgcgggtgccaatgtgcgtcagtttatgtccaagcacgat 15 gaagcgctggtgaaaatgattgaggattacgaggctgatagggccgatcgtgagctgcgctcctttgttctccaggtggacgtaga tacgga att ccattcg a aget cattgg tegt cattgg cgctgtg attaa caaget geg tgccg at cacga cgt cat catttcg ctgcct attagg tegt cattgg tegt cattaagcgggatgaacccaatgaccgcatcatctctatcaccggctaccaggccaatgcggaggcagcccgcgatgccatcctagagattgttggcgaccccgagacacttcatcgcgaggttatcgagatcgataaacgcatccaccccacctcattggccaacgccga cgcaccattcgcaagatcatcgaggataataaggtgaacatcaagttctcagctgatgatgacaaccccaattcgatcttcatcagt 20 ggcaagatagaggacgttgagaacgtcaaggagttgctcttcggcatggctgaggactacgagcgtgactacttggataacgtg gcgatagcgccgccaacgattggtgccttcctaactgggttctggatccgatgccgcaggtgccagcgagaacggattcgtcatc aaagacgcaccgtgggagaagcaaaagcaggccaaaaacctgactgcgcccaacactcagtcgcaggaggacttcccgcact tcgctgctgcggggctccggttgcctccacgcctatcactccgtgtggggccccaaaaactaagtgcatcgggccgagtgtc ccgatcgccagcagtagcagcaatactacaagtcggggtgcgccggggatcggagctggagatggaccaggagctggagca gaagctggaactggaacttgaattggattatcgggcaatgagcggcagagcagcagcagcagcagtcgtgcggacatctctttag 25

Drosophila Gene Hit BLASTN with rescue sequence 1: dodeca-satellite protein 1 (DDP-1) (AJ238847). TBLASTN with ORF2:dodeca-satellite protein 1 (DDP-1) (AJ238847).

30 *Drosophila* EST GH20785 (AI389573), LP07358 (AI294065)

Annotated Drosophila genome genomic segment AE003799

Annotated Drosophila genome Complete gene candidate CG5170 - Dpi dodecasattelite

DNA binding protein CG5576 - Bg5 involved in cytoskeleton organization and biogenesis which is putatively a component of the plasma

40 membrane

Human homologue of Complete gene candidate CG5170- 4885409

ref|NP_005327.1|pHDLBP| high density lipoprotein binding protein

>gi|2498434|sp|Q00341|HB

45

184

CG5576- 2e-07 4506539 ref|NP_003795.1|pRIP| UNKNOWN >gi|3426027 (U50062) RIP protein kinase [Homo sapiens]

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Putative function

CG5170: DNA binding protein (homology with Scp160p, a new yeast protein associated with the nuclear membrane and the endoplasmic reticulum, is necessary for maintenance of exact

CG5576: death domain containing protein, possibly involved in

signal transduction

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Confirmation by RNAi

CG5170: Reduced G1 and G2/M peaks indicating fewer

cycling cells and more polyploidy

CG5576: Loss of G1 peak

185

Example 63 (Category 5)

Line ID 40/2

Category 2nd chromosome, small imaginal discs

Reversion NR 5 Map Position 39B

Rescue ID BamH1

Rescue Sequence 1

GTTCGAATTCGTTCGCACTTTATCGATAGTAGATTGCTATTATGGAGCCCACTA
GTAAATTAATTAAATTCCAGACTGATAAAAAGCGATCAACTTTTGTTAATGGGT
TTAANTCTATAATAATNCTTAGTCCAAATTGTNTCAAAGTAGTCGATAATTTAT
AATAACAGTTTTAGATGACCTCTAGGAAATAACTAATTACCCACATNCTTCAA
GAAAGTGTTTNCAATTTGTNCTATAATTAAATAACAGTTGTATTAATTATGTTG

Rescue ID EcoR1

25 Rescue Sequence 2

AACGGGGGCTTCCGCGNCNCCAAAACGCAATNTACCGTTCATGCTGTGAAG CGAAAAGAGTGGTAGCGCCTACCNTGGCATATGTAGTTAAATCCGTGAAAT AAGTGAATAAGAATATGTATGTACTTAATTCGAAAAACCTTTTCGCCGTCAG CACAACGGGTGAACGAGAGAGCGGAAGTGGAGTTTTTTGTGGCGGGTCGTCT

- 30 CGCTCGCACCGCAAANGTCGTCCGTGGCTGCGTGTATGGGTGTGTGGAAAAA GCGTCGAGGTGAATGTGGATTTCTAACCACACCAGCATTGCAAAGACATTGAT TGATATTTAAAGCTGCAGCAGCGAACAAAGCAAATCCTAATTTCGGCAAAGTT TAAGAATAACGAGTGACTGGGGCGCGCGCAATAAGATAAAATTGAAGGTTAT CTGTGTGCGTGTGAGTGACCGTNTACCAGTGTGTGTGTGCGANCGTCCATTGT
- 40 ATTCGGTCCGATGGAA

45

Genomic hit, Accession No. CSC:AC014744

Drosophila EST several including LD46342 (AI544109 BLASTN similar to mRNA L07550)

| | | <i>la</i> genome genomic segment <i>la</i> genome Complete gene candidat | AE003669 e CG8678 - novel with ankyrin homology |
|--|-------------------|---|---|
| 5 | Human homologue | of Complete gene candidate | CG8678 -gi7661580 B69CEC399B56F35C [ref]NP_056425.1[DKFZP434J 154 protein [Homo sapiens] (2.20E-85) |
| 10 | Putative function | Novel protein with ankyrin domains | |
| Confirmation by RNAi Reduced G1 and G2/M indicating fewer cycling ce | | | |

Example 64 (Category 5)

Line ID

Category

2nd chromosome, small imaginal discs

Reversion

NR

Map Position

49C

Rescue ID

BamH1

Rescue Sequence

- TCTCATGNTCAGGGGGCCTTTACNATGTCAAAGAGCAAATTGTCCACAGGGCA 10 AAGCCGCGACCGGCAAACGTGGCCCGCCCACAAAGCGAGCATTTTCACATTTT AACTGTCTGGACATTTTGTAAGTTACACCAAGGCAATGATACCAGTAAAAAAG TGATTGTAGGTGTTTTAATATACAATGTCTCTATTACTGCTTTCCTTTATTCAAA AGCCATGTGTAAGTGTAAGTTCTCGATTTCGGCTAGATTTTGAAGTTCTGCCAT TATCAATTAAAAGTCCAGTTCCTCTATAAATTGGTAATAAAATAGCTCTTTACA GCCAAGTATATGTGCAATTTTGTAAGATTAAANGTCCAAATGTTGTGAACCTT TCCTGGCCCTGAATTTTAAAAAACCATTAAATTGGTCCCATTTGACATTAAATG TTCTATGTACATTAATATGACTTTTTGTGGATGGTTTTATAAACAAGCATTACT 20 ATATTCTAAAAATCAAGGATAAAGGACNAGCTTTACAGGAGGTAACATTCCTA TTGTACGGCTTTATTTTCTTATACCCATAAGAGCATACCACTAGGATCCGTCGA CCTGCAGATCTCTAAAAACTTGCCTTTGCTGGCGTTTTCCATAA
- Genomic hit, Accession No. AC007085 25

- Associated ORF

Genscan ORF1 predicted sequences >21:54:11|GENSCAN_predicted_peptide_3|108_aa MGLVTAAFKLKRKDIQDRYQHDINRICHTRSTAHTAYAHFAEHLLRRSPRQRFVN GKGAALVLILLVSAARQFSGSTGAYKLGNRVGKVEGEQQEYKLQDRTTHFCGN

>21:54:11|GENSCAN_predicted_CDS_3|327_bp

 $\underline{\underline{}}$ $\underline{\underline$ aggtgctgcgcttgtgctcatcctcctcgtttctgcggctcgacaattttctggctcgacaaggtgcctacaaactgggtaatagagttg35 gaaaagtagaaggggaacagcaggaatacaaactacaagacagaacaacacatttttgtggcaattaa

Corresponds to CG8732

AE003836 Annotated Drosophila genome genomic segment 40 Annotated Drosophila genome Complete gene candidate CG8732 - 1(2)44Dea

homology to fatty-acid-Coenzyme A ligase, longchain previously described

spindle/chromosome

188

abnormalities in neuroblast squashes

Human homologue of Complete gene candidate

1e-171 4758330
ref[NP_004448.1|pFACL3]

fatty-acid-Coenzyme A ligase, long-chain 3

>gi|4165018|dbj|BAA371 and

LCFD_HUMAN LONG-CHAIN-FATTY-ACID--COA

LIGASE 4 1e-157

Putative function Fatty acid CoA ligase

5

10

15 Confirmation by RNAi Only wild type profiles observed

WO 01/72774

189

Example 65 (Category 5)

6/7 Line ID

2nd chromosome, small imaginal discs Category

Reversion NR 28E Map Position

> Rescue ID BamH1

Rescue Sequence 1

TATNAATAATCATAGGGCTCTTGCTCTTACGTGTAAGGCCTGCCCCTCTNCCA 10 GTCTATATACAAAGAAAAACACACACACACTGGCACACTGGTGTTCGCATATG CCAAAGCCGAGTTAATTTCACTTTGTTTAATCTATCGTTTTGGTGTTTTTTGCATTT TTTAACCGCGCAAACGGTATTTGCGCGTTTTGCGCCTCTTACTTTGCGATTTAT TGCACCGCTTGGCTGTTTTTGCAATTTCTATCTTGATTTTCATTGGTATTCACG

- CGTAATGTAATTCTTAGCAGCGTGACCGCGCCGATAACGATAAAAAAATACCAC 15 GGGACCAAAAATAAATACCATATGATACCACTTCAGGGAAAAGAAATCCTAT AAAAGGTGTATTTATAATCAAATACTCGGTACTTNTTAATTACTCCAAGAANA ATTAATTTGAAAAAAGGGGTTCCATTATAAAATATATATTAACCGCTTACAC
- ATAATCCCCAAACAAAACAGCGATTGGGATTTAAAAGGTTCTAAGTCCATCAT 20 TATAAAAGATCATTTCCGAAAAACAAAAGAAATAGATTCAAAATTAGGCGAC ATCAGCCCGCTGATAANGATCATAAAAATACAGAAGCTNATTCAGCGAATCA GAAANTCCTACTCGCCACTATCCGAAAACNTNGAAAAAAAATGG

Rescue ID EcoR1 25

Rescue Sequence 2

TGAAAGGTAGCAACAACGTTTCCTTGGAAAAAGCTGTAAATAGTAAACAAAA TTGTCAAGTTAACGAGCCAAAGTTATTAAATAAGGTTCGAGTACGTTGGCATC GGCTGCCCAGGCAGCAANAAAAACAAAGACGCAGTTCAAGATCAGCTGGAC

- ACTTAGAAGANTTTAAGAATTGAAGCACATTNNAAAGAAGANAAACAAGAAC 30 CCCACCAAAAACCCGCGTGCGTTTGTATGTGTGTGTGCCATCAAATTTCCCGC ACTGGGTGAATGTGCNTGCGTGTGTTNTGTGTCATTTAATTTTCCTACCAATAA TCGCCTTCCAAGAAGTGAATACCAGCCGATCCACCGCTAAATCGAAAAAAGTT TNACTCTGGGTTAANTCACTGTTTACGGCTTTTGTGCTATAATTACCTTTCCCG
- TAAGCNGTGGGAANCTAAAANCCAAAACNTNAGAATCCGAATTCCG 35

Genomic hit, Accession No. CSC:AC017934

Associated ORF

Genscan partial ORF1 predicted sequences >22:35:21|GENSCAN predicted peptide 4|128_aa MGTNSGATAGINNKPVGGATGAGVLVGGGVGGANSSIGGVLSNSLGGGSGGLS ISGLNAGGONANVGGMGNVGGDDGGNGMVGGGVNNQQATTPQYTIPGILHFIQ HEWSRFELERSOWDVDRAELQ

45 >22:35:21|GENSCAN_predicted_CDS_4|384_bp

190

Human Homologue TBLASTN with ORF1: very weak homology with striatin,

calmodulin-binding protein (STRN) (NM_003162.1)

Drosophila EST several including LD42534 (AI516610), LD03224

10

5

Annotated *Drosophila* genome genomic segment AE003619

Annotated Drosophila genome Complete gene candidate CG7392 - novel WD40 family

member

15 Human homologue of Complete gene candidate CG7392- SG2N_HUMAN

CELL-CYCLE NUCLEAR
AUTOANTIGEN SG2NA
(S/G2 ... 622 e-178 A cellcycle nuclear autoantigen
containing WD-40 motifs
expressed mainly in S
and G2 phase cells

20

25

Putative function WD40 protein a novel nuclear protein mainly expressed in S and

G2 phase cells that was characterized using autoantibodies from a

cancer patient

Confirmation by RNAi Reduction of Glpeak, more polyploidy

30

Line ID 103/1

Category 2nd chromosome, small imaginal discs

35 Reversion R
Map Position 57B

Rescue ID BamH1

Rescue Sequence 1

40 GATTTCAAAATTAGGCGACATCAGCCCGCTGATAAAGAATCATAAAAAAATACT GAGGCTTATTTTAGCGAGTCAGAGACTCCTACTCGCCAACTATCGAAAACATA GNGAAGATATAGTCGCCAACCGATCTGCCTTCTATAGTGTTGCTTATTGTTGTC CCCTAATCAAATTAATAAAAATCTGCATTAGGCTGCTTCGCCGGCCAGCAACA AATGTTITACACCTACTGTACTTTTCGCAGAACAGAGATCCAAATGCAGGATC

45 GTTTCCATGACTGTACATTTATTCGGATTAGACATTAAATTACACCCTACAGCT ATACATACTAACAGTGAACACGGCAAATGCTTAGCTAGCATTGGGCCACTTTC GTTGACTGCGAATAAAAATGATTGGCCGATGCCTTTAGCAGATTCCTTTTGAT CGAATTACTCGGATGGCTTGTGTCCACCTCTTACAAGAACTCCTCGCACCA

PCT/GB01/01297 WO 01/72774

191 .

ATCGTTGAGACAGTTGTAGCAATCGGATGCTTGGTTGGAGCTGGCGTGGCACA CCTTCTTCATCCAGTCCTTGGACAGNTTCTTGGNCCTTTTCAGNANCAGGATCT GGTCCCAAACGGNGGAAGGCCTAAAACGAATGGNAATTGATCGGTAGCCCTT GACTGGCATTGGTAATTTGCGCACATGGGNGTCATCGGATTTACACACGCACC ATATCGAATCAGCGTCCTTAAGCGTCAACCGAGGGTTTCCCCAATTCCGGCCA GTTCCGTCACCGACTTGGTTGCCATTGG

Rescue ID EcoR1

Rescue Sequence 2

ATCAAAGCGNCTGGGCCCGTGCATCGCCNCAGCGTTCGTCTTAATTAATTAGT 10 GATTGCAAGCGGGTGCAATTATGCACAAAATTACGGACTAATACAACTGCCC GCTTCGCGCTCTCCATCTCCCATCTCCAAATAGTCGTTTGCTCTTCGCACACAA AAGTGTAAACCCTGTGAAAGGTAGCAACAACGTTTCCTTGGAAAAAGCTGTA AATAGTAAACAAAATTGTCAAGTTAACGAGCCAAAGTTATTAAATAAGGTTCG AGTACGTTGGCATCGGCTGCCCAGGCAGCAAAGAAAAACAAAGACGCAGTTC 15 AAGATTCAGCTGGACACTTAGAAGAGTTTAAGAATTGAAGCACATAAAAAAG AGTTTAATTTTCCTACCAATAATCGCCTTTCCAAGACGTGATTACCAGCCGATC CACCGCTTAAAATTGATAAACGTTTTAACTCTTGCGTTACATCAGCTGTTTTAC 20 GGCTTTTTGTGCTATAAGTTACGCTTTTCCCGTAAGCCGTTGGCAACACTAGAA CGCAAAAGAGCATAAAGAATCGCGAGTACCGTANAGAGGAAGAGAGAAGA GAGAGAGATAGAGAGTGTGAGCGTGTGAGTGAGCGGGAATGTGGGGGCGGT TCCGGTGCGAAAAAACGTAGTAGTAGTACATNNAGAGAGTGCGAACGAGAGG GAGGCAGCCAGCGAGTGTCCTGCGACTGCTCCCCCCTTTACCCTCGTCGCTTTT 25 CTATTCGGAAAATTCAATGACCTCATTTGTTTCATGTGCCGAACTTTGCTTTTC TTTCCCAACCTAAAAACGCAAAAAAAAAAAACNCCAAACAGGATATACGTNG GAACANTGANCAAACNANTTCGANAAAACCAACAACNANGGACCGTGCCCTG GGGCNCCTGAAAGGCAAACAGCTGGCNNCAAATCCGGAAAAGGATCNGGAA NAACAGGATCNGCGGGCNCAAGGATCNCCGGAACAGGCAAAGGAAACNCCC GGCNCACNGCACAAGCCNCTGAAAAGCAACNTGAACCAATGGGCACCANTTC CGGGANCCACCGCTGGCATTAAA

Genomic hit, Accession No. CSC:AC017934

35

rest of results as for line 6/7

192

Example 66 (Category 5)

Line ID 65/24

Category 2nd chromosome, small imaginal discs

5 Reversion NR Map Position 48A

Rescue ID BamH1

Rescue Sequence

- 20 AAAATCCTAGTGAGCTTCGTTGTTAGGGCTGTATGACACGAAAGCAAGTTGAA AAGAAACTTTTTTAAAATTATTTGGTTAATTGAGCAGAACTAAAACTATATN AAAATATTTAAGAATNCAGATTAGTGATGTATTTAATAATAATAATAGTAAGAT GTTC

25 Rescue ID EcoR1

Rescue Sequence 2

- 30 ATTAATAAAAACTGCAATAATGTCAAAAAATCTAATTGAGGCAACAAATTAN CAAAGCCATNAAAGCAGGCTGCACTGCGAGAAAATTGTGCCTTTCCACAGAT CTTCTGCTGCAAAGCNAAAGAANGTAAGCAAGTCGGCCANTTTATTNCATTCT TCTCATCTCTTCTTCGCGAATTGGCGCNTANCACTTACAATAATTNATATNA CTTCTTAAATTTCAAANTCCCTTTCNTGAACGGANCTTTTAACGGAAAACAAA
- 35 GCGGGTAAACTAACTAAACTAAACTAATTANAANTGTANGTATAAATGAACC GAACTCGCTTTAGATATNATGCGTTTCACTAACANATTANAACAAACTTTGAA GCTGTANTGTCAGGTTGTTATTNCGTTCACCANATGTAGACTGNCCGNNAATT TNACCTTTCCCAATANTCTGTTCTTAANTGTNTTGTTTTTTCCCAATNNTTTGATC ATNCNTTGGTNAATNANCTNAACGGCCCAAAGTNAATGAATTCCANTCACGTC
- 40 CACTGGCTCTGGTTCNATANTTAATNGGCTGTTTCTTACTTCCCTTAACCCTAA CATCTNTTAATCACCTGTGCCATNTGTTTGTGTGTGTGAACGAATGAGAAA AAAAA

AE003825

193

Annotated *Drosophila* genome Complete gene candidate CG9005 - novel putative cell adhesion

Human homologue of Complete gene candidate CG9005- Ensembl predicted gene

ENSP00000006008

Gene:ENSG00000005238

Clone: AC004472

Contig:AC004472.00001 6.00E-38 (KIAA1539 protein AB040972) and AK022837 Homo sapiens cDNA

FLJ12775 4e-33

Putative function Putative cell adhesion protein

Confirmation by RNAi Reduced G2/M peak

5

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WO 01/72774

PCT/GB01/01297

194

Example 67 (Category 5)

Line ID 74/3

Category 2nd chromosome, small imaginal discs

5 Reversion NR Map Position 47A

Rescue ID EcoR1

Rescue Sequence

30

40

GCACAGAATGGCNCCTTCACGACAAAAGATCTNCNAATTAGGATGATGCAGA AGGAGGACACGCTTTTCATTATCTGGTTGCCACCTAATTTAAGTTCCACATCAA GAAAAGCCTGTCTATAAAAACACGATAACGTTTTTGCTAATCTCAAGACAATG TTAAATATAATTGGAGAAAGTATTGAATATGAATATCACAAAAATTGTTTAGG 15 GTCTCTACGTGGTAAATAGTATTTGGCATAGACAGTGAGATGTGAGTCGTACG TACTAATTAATAAAGTTGTTCAARAGAACCTCATATACTGTAAGTGACAACGA ACGAAGCTGACAACTCTGCTTGCACATATTTGGCGGAGTTCGAAAATATCATC GCATTGGTATTGTTTTGTNTCCACCNTGGGGCGAGATTTTGTTGTTGCTTTAC TTTGCTTGTTTTTCNCCACAAANCGAACCATAATGTTCGAAATGGTAAAATTA 20 CCGTGCCAACAAGCTCTCTCTCCCCACTCCGAAACTCTCTCATCTCCTTG CAATTGTTTAAGGTGTGCAAGGAAATGAAAAATGTCCCGGCTGTGTTNCCATG CATTCCCCTTCAAAGCCAATTATNTTTGTGCCTCTCCAACNTTTTTGATCGGNN TGATTTTTTGGCTCCCCNTANTCCCCCCCCCTTTCNCCCATTCCGGGTTANAT TATTNTNCCAATTTTCCTATTTTACGGTCCCNGTTCCCTGGAAATANTTCCTNC

25 AATCNCCGCTCCATNTCNCCATNTTTGACAGATTTTC

Annotated *Drosophila* genome genomic segment AE003829

Annotated *Drosophila* genome Complete gene candidate CG12052 lola -a specific RNA polymerase II transcription

factor involved in axon

guidance

Human homologue of Complete gene candidate 1e-09 3789797 (AF059569)

actin binding protein

35 MAYVEN [Homo sapiens]

Putative function lola-like specific RNA polymerase II transcription factor

Confirmation by RNAi Almost no G1 peak and increase in G2/M peak indicating

arrest in G2/M

PCT/GB01/01297

Example 68 (Category 5)

WO 01/72774

79/7 Line ID

2nd chromosome, small imaginal discs Category

5 Reversion R 55B Map Position

> Rescue ID BamH1

Rescue Sequence 1

GTCTCATGCACCCTGGCCCTNAGCTGCATAAGTGTAAGTGTGTGNCTGTGTGC 10 GAGTGTGGGTAGGCGGCGCAACTATCTCGCTTGCTCTTGCGTCCGGGGTTAT CGGTAGCTTCTTCTAGGCTGAGTGCATTTCGTTGAATCGTGGATGTTGAAAGTT GTCTAATTTCCGAACTATTGATTTTTCCCCTTCCCCGTCAAGAAACTGCATTGT TGCTTCTTGAAGACCAGTTTTGGTAACATCAGGAGAATGGAAAGGAGCGAGT

- GAGTCGGTGAGTAAGTGAGTGAGCGATGCGAGCGACAAAATCAACAACAACA ACAACAACGGTCAAAACGAGTTCCAACGAAAGTTGCAACACTCTCAACAATT TGAGCAGCTCCGTTTGTTGTTATTGCATTACTCAATCGGGAAGACTCTACACTC GACGGAATAGTGTGCTCGTCTGAAATTTATCNATTTCCATTCCTTCCTTTGTTT TTGGGCCAAACAATGGCNTCGGCAANCGTTCGTGGAAAACCGCAGGAACCAC
- CAAAATGCCTGGCGTCACATTAACCGAGCCGCCTTTGTTTATGCAAATATTATT 20 GTAATATTTGGTNAAAATTAAGTCGCGCTTCNCGTTACTTTTATTTCATATAC ACGCAGCAGCACGCATACAGTCACGTCACGCACACATACAATCGCCGTN CACATACACTTGTCTTTTTNCCACACACTTTCCTAATCAT

25 Rescue ID EcoR1

Rescue Sequence 2

NGGNGTCTCATGCACCCTGGCCCTNAGCTGCATAAGTGTAAGTGTGTGNCTGT GTGCGAGTGTGGGTAGGCGGCGCAACTATCTCGCTTGCTCTTGCGTCCGGGG TTATCGGTAGCTTCTTCTAGGCTGAGTGCATTTCGTTGAATCGTGGATGTTGAA

- AGTTGTCTAATTTCCGAACTATTGATTTTTCCCCTTCCCCGTCAAGAAACTGCA 30 TTGTTGCTTCTTGAAGACCAGTTTTGGTAACATCAGGAGAATGGAAAGGAGCG AGTGAGTCGGTGAGTAAGTGAGTGAGCGATGCGAGCGACAAAATCAACAACA ACAACAACAACGTTCAAAACGAGTTCCAACGAAAGTTGCAACACTCTCAAC AATTTGAGCAGCTCCGTTTGTTGTTATTGCATTACTCAATCGGGAAGAACTCTA
- CACTCGACGGAATAGTGTGCTCGTCTGAAATTTATCNATTTCCATTCCTTCCTT 35 TGTTTTTGGGCCAAACAATGGCNTCGGCAANCGTTCGTGGAAAACCGCAGGA ACCACCAAAATGCCTGGCGTCACATTAACCGAGCCGCCTTTGTTTATGCAAAT ATTATTGTAATATTTGGTNAAAATTAAGTCGCGCTTCNCGTTACTTTTATTTC ATATACACGCAGCAGCACGCATACAGTCACGTCACGCACACATACAATCGCC
- GTNCACATACACTTGTCTTTTTNCCACACACTTTCCTAATCATNNTA 40

Genomic hit, Accession No. AC004296

Associated ORF

Genscan: ORF2 predicted sequences >15:31:31|GENSCAN_predicted_peptide_3|109_aa 45 MVTSFRHLRDEKSFTDVTLACEGQTCKAHKMVLSACSPYFKALLEENPSKHPIIIL

KDVSYIHLQAILEFMYAGEVNVSQEQLPAFLKTADRLKVKGLAETPSSIKREG

>15:31:31|GENSCAN_predicted_CDS_3|330_bp
atggtgacctcgttccgtcacctgcgcgacgagaagagcttcacagatgtaacactcgcctgcgagggccaaacctgcaaagcc
cacaaaatggtgctttccgcttgcagtccctactttaaagcgctactggaggagaacccatcgaagcatccgatcattatcctgaaa
gatgtctcctacattcacctacaggctatactggagttcatgtacgccggtgaggtgaacgtgtcccaggaacaattgccagcattt
cttaagaccgccgatcgcctcaaagtgaaaggcctcgcagagacacccagttcgataaagcgggaaggttga

Drosophila Gene Hit** TBLASTN with ORF2: several zinc finger proteins including
Broad-Complex mRNA for BRcore-Z2 protein (X54665)

Human Homologue TBLASTN with ORF2: kelch (*Drosophila*)-like 2 (Mayven actin binding protein) (KLHL2) (AF059569)

| | Annotated Drosophila genome genomic segment | AE003800 |
|-----|--|-------------------------------------|
| 15 | Annotated Drosophila genome Complete gene candidat | |
| | | putative kelch-like putative |
| | | specific RNA polymerase II |
| | | transcription factor known to |
| | | affect disc morphology |
| .20 | | * |
| | | or could be CG10914 - novel unknown |
| | | unknown |
| | Human homologue of Complete gene candidate | CG5738- 9e-09 3789797 |
| 25 | Truman nomorogue of Complete gene canadance | (AF059569) actin binding |
| | | protein MAYVEN [Homo |
| | | sapiens] |
| | | |
| | | CG10914- predicted gene |
| 30 | | ENSP00000051207 |
| | | Gene:ENSG00000047313 |
| | | Clone: AC068261 |
| | | Contig:AC068261.00019 |
| 0.5 | | 4.00E-49 (potental cell |
| 35 | | division GTP binding protein |
| | | 1: ENST00000051207 |

Putative function CG5738: lola like specific RNA polymersae II transcription factor, CG10914: Possible GTP binding protein

Confirmation by RNAi Both show marked reduction in G1 to G2/M ratio

Example 69 (Category 5)

Line ID

80/2, 81/8

Category

2nd chromosome, small imaginal discs

5 Reversion

R

Map Position 57D/E

Rescue ID

BamH1

Rescue Sequence 1

- 10 CANTTTCAGAGGCCATAGNCCTTCACAAAATTCNCCATCTCTGCCCGGCATCC
 GTGCTTGAAAATGGTGCCAATGCGTCGTGGAGAATCTGCTGCACTCGATGGTC
 TGCAAAATTGCACATTTATTAGATTTAATAAATTTTTCAACTGTCCGCGANCAC
 GTTTGCTCGTGTTGAATTTCGAGTACAAAATTAGTGCGACTGTTGGATTGCATT
 GAAATGCCAAAAATCGGTGTGACCATTTCGAAGTCCCCACAGGCTCATGACTT
- 15 TCGCGGTTCACCAAATCCAAATAACGCAAGCTGGTCACGCTGTCAAACATCGG
 TGACGGAATGGTGACGACACAAACAATTTGCTTAAAAACTTTCTTGCGGCCGT
 AAAAATGCGCAAGCAGCCTGGCAGCGCAACGCACGTACACGTAATTGGAACA
 AATGTTTGCTGAACCACAACCGCCCACTAAATGTTANCCGCCAAGTCTTTTCC
 CCCGCCGCCGCCGTCNTCNTCNTCNCCGGATTATTTGGTTTACAATTTGCTTAC
- 20 ACAAGTGCAATCGTCGATAGCGCTTCATTTTGGAGTAACAAGTAATATTTTGC GCCGTACTGCTGTTCGCCGTATCAGACAGAAGGTTGGTATCAGTTCGACGCAG CTTGTGACGGTATTGCATACGCGGCGAAACGCCCACGTGAAAACGGATCGCA GTTCTCGAAAACTCNGGATAAAAA
- 25 Rescue ID EcoR1

Rescue Sequence 2

TGGGGTCTCANGCCCCGACGCCATATTTTAACACAAGATTCNNCANCTCTGC AGGGCATCCGTGCTTGAAAATGGTGCCAATGCGTCGTGGAGAATCTGCTGCAC TCGATGGTCTGCAAAATTGCACATTTATTAGATTTAATAAATTTTTCAACTGTC

- CGCGAGCACGTTTGCTCGGTGTTGAATTTCGAGTACAAAATTAGTGCGACTGT
 TGGATTGCATTGAAATGCCAAAAATCGGTGTGACCATTTCGAAGTCCCCACAG
 GCTCATGACTTTCGCGGTTCACCAAATCCAAATAACGCAAGCTGGTCACGCTG
 TCAAACATCGGTGACGGAATGGTGACGACACAAACAATTTGCTTAAAAACTTT
 CTTGCGGCCGTAAAAATGCGCAAGCAGCCTGGCAGCGCAACGCACGTACACG
- TAATTGGAACAAATGTTTGCTGAACCACACCGCCCACTAAATGTTAGCGCCA
 ACTNCTTTTCCCCGCCGCCGCCGCTCGTCNTCNTCCCGGATTATTTTGTTTACA
 ATTTGCTTACACAAGTGCAATCGTCGATAGCGCTTCATTTTGGAGTAACAAGT
 AGTATTTTGCGCCGTACTGCTGTTCGCCGTATCANACAGAAGGTTGGTATCAG
 TTCGACGCAGCTTGTGACGGTATGCATACGCGGGGAAACGCCACGTGAAAAC
- 40 GGATCGCAGTNCTCGAAACTCNGGATAAAAGAAAAAGTAGGCTGAATG

Genomic hit, Accession No. AC007175

Associated ORF

45 Genscan: ORF2 predicted sequences >16:09:09|GENSCAN_predicted_peptide_3|2497_aa MNEGNSAGGGHEGLSPAPPAVPDRVTPHSTEISVAPANSTSTTVRAAGSVGAALP

ATRHHQHIATQVKGIASSSSKQQKQLASAQLPVPLSPLPQQQQQTAEATAAAAAP AHSNVSVSSSTIEASVLPPQAKRQRLDDNEDRTSAASIVGPAESSNIVSSLLPASVA SSSEVGGLSSTALQDLNALKKRILQQKLQILRNLKERHLENVSEYFYLQNGGSMM DYPAWRKKTPTPQFISYSNANRIDQLIHEDKPSTSAAAAAAQNQKYTTQQTDSVE SSLVSGIGTGATKGAPLDGNISNSTVKTNTQSQVPSKIGSFTESTPAATESNSSTTVP GTATSGAATSTSATSAEASGNVLAVEAEIKIPAVGATPVAISTKLPAAVVQLTQQG GTPLLPCNTSAGSTALRRPQGQNNASSGSAAASGGGGSLTPTPLYTGNGPAALGG SGGLTPGTPTSGSLLSPALGGGSGTPNSAAQEFSFKAKQEVYVMQRISELQREGL WTERRLPKLQEPSRPKAHWDYLLEEMVWLAADFAQERKWKKNAAKKCAKMV QKYFQDKATAAQRAEKAQELQLKRVASFIAREVKSFWSNVEKLVEYKHQTKIEE KRKQALDQHLSFIVDQTEKFSQQLVEGMNKSVADTPSLNSSRLTSPKRESDDDFR PESGSEDDEETIAKAEEDAADVKEEVTALAKESEMDFDDFLNDLPPGYLENRDKL MKEEQSSAIKTETPDDSDDSEFEAKEASDDDENTISKQEEAEQEIDHKKEIDELEA DNDLSVEQLLAKYKSEQPPSPKRRKLAPRDPELDSDDDSTAVDSTEESEDAATED EEDLSTVKTDTDMEEODEOEDGLKSLMADADATSGAAGSGSTAGASGNKDDML NDAAALAESLQPKGNTLSSTNVVTPVPFLLKHSLREYQHIGLDWLVTMNERKLN GILADEMGLGKTIQTIALLAHLACAKGNWGPHLIVVPSSVMLNWEMEFKKWCPG-FKILTYYGSQKERKLKRVGWTKPNAFHVCITSYKLVVQDQOSFRRKKWKYLILD EAQNIKNFKSQRWQLLLNFSTERRLLLTGTPLQNDLMELWSLMHFLMPYVFSSHR 20 EFKEWFSNPMTGMIEGNMEYNETLITRLHKVIRPFLLRRLKKEVEKOMPKKYEHV ITCRLSNRQRYLYEDFMSRAKTRETLQTGNLLSVINVLMQLRKVCNHPNMFEARP TISPFQMDGITFHTPRLVCDIMEYDPFTQINLETLNLLLHLEQTMTAYVSHKSRLL APPRKLIEDIDTAPLPAPRCPNGKYRFHIRVRSAELAQRIKLNAVKVGASPAMRLE GSKIMPMRNLLPSGRVLKRVSASINPVNMALKPVVINSVVTTTSSSTTASSPTGAL 25 SVLSNSKLLGARSQINAPTPAKVAKTMQDGKPFFYLTPATNSGAAGARLTLTSKT TASASTTTSRTTVTASTTSGQQLIRDPIVKDLATHVKSTVQKQSIANGKTEPEEETE AEDPYKVQELIQMRKEQRLAALKRMAMINRRRTDATPIYGEDCREAIORCMOAT RSLKRSTWQTRGYANCCTAMAHRNGWSLNHLLKSFEERCADLKPVFANFVIYVP SVCAPRIRRYVONLSSTHWOHEORIENIVDOALRPKLALLHPIISEMTTKFPDPRLI 30 QYDCGKLQTMDRLLRQLKVNGHRVLIFTQMTKMLDVLEAFLNYHGHIYLRLDGS TRVEQRQILMERFNGDKRIFCFILSTRSGGVGINLTGADTVIFYDSDWNPTMDAQA QDRCHRIGQTRDVHIYRLVSERTIEVNILKKANQKRMLSDMAIEGGNFITTYFKSS TIKDLFTMEQSEQDESSQEKSENKDRIVATTTLSDTPSTVVETEKQSLRAFEHALA AAEDEQDVQATKTAKAEVAADLAEFDENIPIATEDPNAEGGPQVELSKADLEMQ 35 NLVKQLSPIERYAMRFVEETGAAWTAEQLRAAEAELEAOKREWEANRLAAMHK EEELLKQETEAEEMLTYSRKDSSNQVNTKTDSNSNKRRLVRENRRNSAQKLSRSV SSHSTGSNNKNSKSATTRGNSQNSLNQTVPVGSGISRVNRTGAGVSSSSRGKSNST KSTGKGTDAAPQVRRQTRLHSLGAVNMASARTPPTRKTTRTALAASAAASTLED ASLIVEERPKRQSANIAMSKMMKTPFKQNVPSNISIKTTPPKRGRRDSVAAAATRS 40 KLLERRATIAAPLKHMDDESDQDEEEQEEQESEEDTEGEEANATVDDDEEGEEEL ASLDEETIQTGSQTNDEEDDDEEEVGEEGMVDIDTEDSEADVKSSSTYGTAADGK PEEAESLDGWDAHDQVQDTTMTSSTYYNVSEESDTDEHHDSKAEAKEPPONSDK SDESEAVGHTPRTRSRGTVKINLWTLDVSPVANALNKSSANRSLKKAPRTESTPK ESQSEPRRKITQPKLPKKEETNNKSNSNIGTLHRWISKSPRVMLRSTPVTAASASSS 45 AAVSGVSGGNASSSGTAR

>16:09:09|GENSCAN_predicted_CDS_3|7494_bp atgaatgaaggtaattcagcaggaggggggatgaagggctcagccggcccctcctgctgtgccagaccgcgtaactccaca

199

tt caacggaa at tt cag tt gcccccgccaatt ctacaa gcacaa cag tacgag cag cag gat cag taggag cag cctt gccggccccc and the companion of the companioaccegccatcaccaacatatagegacccaagtgaagggaategccagcagcagcagcaacaacagaagcaactggccagtg 5 gaggacaggacgagtgccgccagcattgttggaccagccggaggagcagcaacattgtaagctccctgctaccagcgtcggtggc ctccagcagcagggctttcttctacggccctgcaggacttgaatgccctcaagaagcgcatactccagcagaaattgcagatettgegtaatettaaaggaaaggeatettgaaaatgtgteegaataettttaeetacaaaaeggeggeagtatgatggaetaee ccgcgtggcgcaagaagacaccaacccgcagttcatcagctacagcaatgcgaatcgtatagatcagctgatacacgaagata 10 gtcagtggcatcggtactggagcgacaaaaggagcgccattggatggcaatatcagcaatagtactgtgaaaacgaatacgcaa tetcaagttecaagcaagattggcagetteacagaatcaacgcccgcagcaacagaaagcaactcaagtaccacagttecagga a cagcta caagt gg eg eg caaccag cacat cagcta ctt egg ecg agget ag t gg taat gt ect gg eagt gg aag cagaa at each effect of the second entropy of the secoaaaatcccagctgttggagccacaccagtggccatttccaccaagcttcccgctgccgtcgtccagctaacgcaacaaggtggca cccctttattgccctgcaatacatccgccgggtccacggcgcttcgtcgtccccaaggtcagaacaatgcctcaagcggatccgc 15 cgcggcatctggaggcggaggaagcctcacacccacaccgctctacactggcaatggcccggccgctctgggcggtagcgga ggactcacgcctggcactccaacttctggcagtctgctcagccctgccttgggcggtggctccggaacgcccaacagtgcggcg caggagttctcttttaaggccaagcaggggtatgtgatgcagcgtatatcggaactacagagaggggattatggactgagc gattttgcacaggaacgcaagtggaagaaaaacgcggccaagaagtgtgccaagatggtgcagaagtatttccaggacaaggc 20 ttggtcgaatgttgagaagctggtcgagtacaagcaccaaactaagatcgaggaaaaacgcaagcaggctttagaccaacacctcagetttattgtagaecagaeagaaagtteteaeageaattggtagagggaatgaaeaagagtgtggeggataegeeeagtetta attotagccgtctaacatcgccgaaacgggagtccgatgatgactttcgccctgagtctggttcagaagatgatgaggagactatc 25 ctta at gate tacca cct gg ctatct gg aaa at cgt gataa gct tat gaa ag ag cag ag ct cgg cgataa ag acc gaa ac gc consideration of the cona caa g tot gaa caa acctect a g to caa g c gaa g a a g tot gaa caa g tot g tot gaa caa g tot g tot g tot gaa caa g tot gagttgattccaccgaagaaagcgaagatgcggccaccgaggatgaagaagatctctctactgttaaaactgatacggatatggag 30 gaacaggatgaacaggaggacggtcttaagagtctaatggcggacgctgatgcaacaagtggtgctgctggcagcggaagcac ggctggggcaagcggcaacaaggatgatatgctgaacgacgctgccgccctggccgagagcctccagcccaagggtaatacc ttgtcctcaaccaatgtggttactcctgtgcccttcctgctaaagcactccttgcgtgagtaccagcacatcgggctcgattggctggt caca at gaat gag c gaa ag t taa ac g g cat ctt g g cc gac gag at g g g caa g ac cat c cag ac cat t g c g ct at t g g caa g ac cat cc ag ac cat t g c g ct at t g g caa g ac cat cc ag ac cat t g c g ct at t g g caa g ac cat cc ag ac cat t g c g ct at t g g c caa g ac cat cc ag ac cat t g c g ct at t g g c caa g ac cat cc ag ac cat t g c g caa g ac cat cc ag ac cat t g c g caa g ac cat cc ag ac cat t g c g caa g ac cat cc ag ac cat t g c g caa g ac cat cc ag ac cat cc accaccttgcctgcgcaaagggcaactggggacctcatctcattgtggtgccttcgtctgtgatgctcaattgggaaatggagttcaa 35 cctggatgaagcgcagaacattaagaactttaagtcccagcgctggcagttgctacttaacttttccacagagaggcgtctgttattattaaggaatggttctcgaacccaatgactggcatgattgagggcaacatggagtacaacgagactttaattactcgtctgcacaagg 40 tgattogtccgttcctacttcgacgcctcaaaaaggaggtggaaaaacagatgcccaagaagtacgagcatgttataacgtgtcgt cgtgataaatgtactgatgcagttgcgaaaagtgtgcaatcatccgaacatgtttgaagcgcgtcctacgatctcgccatttcaaatgctcttgctgttgcatttggagcaaactatgaccgcctacgtctcgcacaaatcccgcctgctcgccccgcctcgcaagctgatcgag45 gatatcgatacggctccattgccagctccccgttgtccaaatggcaaataccgctttcatatccgagttcgtagcgctgaactggcg cagcgcatcaaattgaatgctgtgaaggtaggagcaagtccagccatgcggttggagggttcaaagattatgccaatgcgcaattt gctacca agtggaagagtgctgaaaagggtcagtgcttcgatcaaccctgtgaatatggctttgaaaccagtggtgatcaatagtgt

gttcacaaattaatgctccaacgcccgctaaagtagcgaaaacgatgcaagacggaaaaccatttttctacctcacaccggcgac gaattcaggagcagcaggagcgcgtcttaccctgacaagcaaaaccacagcctcggcgtccacgacgacctccagaacaaca gttacagcatcaactacttctggtcagcaactaataagggatcccattgtcaaagatttggccactcatgtaaaaaagcacagtacaa aagcaaagcattgccaatgggaagacggagcccgaggaagaaactgaagcagaggatccctacaaagtacaggagctgattc agatgcgcaaggagcagcgattggcagcgcttaaacgtatggcaatgataaatcgtcgccgaacggatgccactcccatatacg gcgaagattgtcgcgaggctatacagcgctgcatgcaggcgacccgatccctaaagcgatcaacctggcagacgcgtggatac gccaactgctgcactgccatggcgcatcggaacggttggtccctaaaccacttgctgaagagcttcgaggaaaggtgcgctgatc taaagccagtgtttgccaactttgtgatctacgttccttctgtttgtgcgccccggatccgtcgttatgtacaaaatctctcatcgacgc actggcagcacgaacaaaggattgaaaacattgtggatcaggccctgcggcctaagctggcgttgctgcatccaatcatttcgga aatgaccactaagttcccagatccgcgtctcatccaatacgactgtggcaagttgcagaccatggatcgtttgctacgccagctaaa 10 ggttaacgggcatcgtgtactgatattcactcagatgaccaagatgttggatgttttggaagcttttctcaactaccacggtcatatttat ctgcgtttagatggctctactcgggtggaacagcggcagatcctgatggagcggtttaatggagataaacgaatcttctgcttcatc ctctccacgcggtctggtggagtgggcatcaatttgacgggtgccgatactgtgatcttttacgactccgactggaaccccacaatg gatgegcaggcccaagategttgccategtattggtcaaaegegagatgtacatatctaeegtcttgtctccgaaagaaccatagaggttaacattcttaagaaggcaaaccaaaagcgaatgctgagcgacatggccatcgagggtggcaactttacaactacgtacttta 15 agaattgttgctacaacaacgctttcagatacgccttcgacggttgtggagacggagaagcagtcactgcgtgcatttgagcacgc gttggctgccgcgaggacgagcaggatgtgcaggccacgaaaacggctaaagccgaagtggcagctgatctggccgagttc gacgagaacattcctattgcaacagaagatccaaatgcggaaggaggtcctcaagtggaactcagcaaggccgatctggagatg cagaacttggttaaacagctctcaccgatagagcgatatgccatgcgctttgtggaagaaactggagcagcatggacggcggaa 20 caattgcgagccgcggaagcggagctggaggcccagaaacgcgagtgggaggccaatcgcttggcggccatgcacaagga ggaggagctgttgaagcaagaaacggaagcggaggagatgcttacctacagtcgcaaggattcgagtaatcaggttaataccaa a a cagatte ca atte ca ata agega cga ctggt gaggga aa ateg cagaa acteag ctcaga aget gag cagga gtgt tag cagaa acteag ctcagaa get gag cagga gtgt tag cagaa acteag ctcagaa acteag acteccatagcaccggtagcaacaacaagaacagtaaatcggcaacgacccgtggaaatagccagaacagcctcaatcagactgtac 25 agtea acgggaa agggaa caga cagcaccgcaa agttcggcggcagacccgtctccactctctgggcgcagtcaatatggcgcagaccgcagaccgcagaccgcagaccgcagaccgcagaccgcagaccgcagacagaccgcagacagaccgcagacagaccgcagacagaccgcagacagaccgcagacagaccgcagacagaccgcagacagaccgcagacagegceegaacaeegceeactagaaagaeaaeaegtaeagetetggetgeatetgeagetgeatetaetttagaggatgeetetttgatcgtcgaggagcgtcccaaaagacagtcggccaacatagctatgagcaagatgatgaagacgcccttcaaacagaatgttctggaaagaagagctacaattgctgctcctttaaaacatatggatgatgaaagtgaccaggatgaagaggagcaggaagaagagcagg 30 cttgacgaagagaccatacaaaccggatcgcaaacaaatgatgaagaagacgatgacgaggaagaagttggtgaagagggaa tggttgatattgatactgaagattcagaggcagatgtcaaatccagctccacctatggtacagcggcagatggtaagcccgaagaa gccgaaagcttggatggctgggatgcacacgaccaggtgcaggacaccacaatgactagctccacctactacaatgtcagcga ggaatcagacacggatgagcatcacgatagcaaggcggaggctaaagagccgccgcaaaattccgataagagcgacgagag 35 cgaggctgttggacacacacacacgtacaaggtcgcgcggcacagtaaagatcaatctgtggaccctggacgtgagtcccgtagc aaacgcattgaataaaagcagcgccaataggagcctcaaaaaaggacccaaggactgagtccacgccaaaggagtctcagagc gtggtgtttcgggaggaaatgcctcctcgagcggaacagccaggtga 40

Drosophila Gene Hit TBLASTN with ORF2: brahma protein (M85049) and imitation-SWI protein (ISWI) (L27127) and chromodomain-helicase-DNAbinding (CHD-1)

Human Homologue

BLASTX with EST TBLASTN with ORF2: Snf2-related CBP activator protein (SRCAP) (AF143946) and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) (NM 003072.1)

201

several including SD07794 (A1534784), LD34465 (AA990657) Drosophila EST Annotated Drosophila genome genomic segment AE003453 Annotated Drosophila genome Complete gene candidate CG9696 - domino an enzyme 5 involved in DNA repair homology to snf2 family helicases CG9696- gi4557447 Human homologue of Complete gene candidate 10 416409C913D6A935 |ref|NP 001261.1| chromodomain helicase DNA binding protein 1 [Homo sapiens] (1.90E-85 15 **Putative function** snf2 helicase family member protein that contains a chromodomain, which occurs in proteins that are implicated in chromatin compaction, and an SNF2/SWI2-like helicase domain, which occurs in proteins 20 that are believed to activate transcription by counteracting the repressive effects of chromatin structure Confirmation by RNAi Loss of G1, peak, increae in G2M indicating arrest in G2/M

202

Example 70 (Category 5)

Line ID 99/31

Category 2nd chromosome, small imaginal discs

Reversion NR Map Position 53E

> Rescue ID EcoR1

Rescue Sequence 1

10 AAGGCCCGACCAGAAACGAAATTTTCGGCGCGTNTTTTAAAATGCGCGGTAA TTGTGTGTTCGCCTGGCTTTGCCTTTTAATTTTTATTTACCTGCATCCGATTCG GTATTTGAAACAGCCGTTGAGTCTCCTTTGGCTTTTTTATCAGCGACGTCATCA GTGGCGCAGAAGCAGAAGCGTCGACAGCGGCGGGGATTCGGCTGCATCTT TGGAGCCCCTTTCCGGCTGTGCCCCCACGGCTTTCGCCACCCCCGCAGTAACC 15 GATGCATTTTCCACATCGCTTACCTTATCGGCGGCATTTTCTTTGGCTGCCGTT TCTGCCGCTTTGTTAGCATCCTTTTCGTGCGGCGANGGCATGGAAAGATACAA ATCAGAATTGGATTACACTTGCTAATTTTTTGGCGGNCAATACAATGGTTCGG TGCGCCTATTCTTTTTAATCGAATCGCAATTGAGTGTNAATTAAGTCTCCGCA 20 ATGCAATTTGTGTATCTGTCTCCCCCGANCGAACAACGATNGAAAAAGGAA CCAGAAATAAAANAGGNAATGAAAAAACACATTGCAATCTATAAGGCCACAC ACACACATATCATCCGTCTACCANTCCATCGGATTCGANCCACANAANCCAT NTTTATACCNCAACGAACGNGGAAAAAACNATATCNGNAATTACCCCCCGAA AATTGTTGCCNCTTTTACCCAAATATTTACAACCNCCGTTCATTCACTCCTGGA

ACATTCCNGGCTTTCCCAATTTTCNCCTTTACTACAATTTCAATGGTTTCTTTTT

Rescue ID BamH1

Rescue Sequence 2

CCTCAC

30 CCTNAAATGTNGCGCTGGGNCCTAAANCGTCNCTCCTTGTGTCTCTCTTGTTTA CCGCGCTATGCTGATGTTGGCATGTTCGATCCCCCTCCGTGTCGATGTTTA CCTTCCTTGGCTTTTGTTCATGCTAAATCCTTTAAATGGGGTTCTGCGTAGTTT AATGCCGAGGTACAGCAAAACTTCAATATTCATGTTCCCTTGCGCTCCCAAAC GAAATTAGCATTGGACGTCCCAAGGTTGAAGACATTTNATTATTTTAACATCT 35 TTTTNATTTIATTACATTTGAACTCTTACAAGTAATAATAATTACAATTAATAT TATAGCTGCAGCGGACAAAAAGGAGAAATCCCCCTCGCCGGTAATAAAGAAT CCAACAATAAGGATGCTNAAAANGAAGAAAACCCNAAAAAGGAGAAGAAAA

40 TGAAGAGAGCGAGAAGAAGAAANCGANATGAGATGCAGAGGACAGATAAAG GATGCCACNGATGAATCCAAGCCAAAATCGGGAGCCGATAAGCCCAAGAAAC TGAGCCCAAGGCCAAGATGGCAAGGTGGNT

ATCGGAANAAGGNGATGAGCCNGAAGATGAGGNNGATGAGAAAGCTAGCGA

Genomic hit, Accession No. CSC:AC020063

45

25

Associated ORF

203

Genscan ORF1 predicted sequences >16:48:25|GENSCAN_predicted_peptide_1|722_aa MPSPHEKDANKAAETAAKENAADKVSDVENASVTAGVAKAVGAQPERGSKDA AESPAAVDASASAATDDVADKKAKGDSTAVSNTESDAAAADKKEKSPSPVIKKS NNKDAKKEDNSEKDEENSEDGDEPEDEADEKASDEESEKKKPKLDAEDKIKDAT 5 DESKPKSGADKPKKPEPKAKNGKVAKEEDDDEEDEDDEDAEDDDGDENDGLDK NNEVAEDDENVVALAEIDRINENINKTRVDGLQTLHAICFGAQGKNNVVKKNLRS FAGFEFAKDSAEYNKKLEAIKKVDNKGLRSICEILTLDRKGSKNETVLRVLKFLM EPDESLCLEQGDEEEEEDAEDEDLDEDEEDPPSEEDKKRKSGKSSGGAGRGSARN STGRPRRATAGKKMSAYVDFSSSDDSEQKVAVPKRRRNDDSESGSDYNPSANSD 10 SDGGRGGGAGAAGRKVPSRGGRGRPARKSRRNSDSEEEEESEVSDADSDVPKR KRGSVGKRGRPAAPASAGRRGRGRGAASRKRKDSDSEDEEVSEDEEEEDVSDFA SDQSEVCKFNLISSIWCFIKYMPIFQEERPKKSKKPITPAKNSKANNKSKPAGKADS RSKKSKKESSEEDDDVDDKDESDEDEPLTKKGKOAFPTDEQIRGYVKEILDKANL EEITMKTVCKQVYAKYPDFDLTDKKDFIKATVKADGVQDLDGSPELIPRGRTTVT 15 **IWLICCCNNQIFGET**

>16:48:25|GENSCAN_predicted_CDS_1|2169_bp

atgccatcgccgcacgaaaaggatgctaacaaagcggcagaaacggcagccaaagaaaatgccgccgataaggtaagcgatg tggaaaatgcatcggttactgcgggggtggcgaaagccgtgggggcacagccggaaaggggctccaaagatgcagccgaatc20 $\verb|ccccgccgctgtcgacgcctctgccgccactgatgacgtcgctgataaaaaagccaaaggagactcaacggctgtttca||$ aataccgaatcggatgcagctgcagcggacaaaaaggagaaatccccctcgccggtaataaagaagtccaacaataaggatgc taaaaaggaggacaactccgaaaaggacgaggagaactcggaagacggcgatgagccagaagatgaggctgatgagaaagc tagcgatgaagagagagagaagaaaccgaaattagatgcagaggacaagataaaggatgccactgatgagtccaagcca aaatcgggagccgataagcccaagaaacctgagccaaggccaaggatggcaaggtggctaaggaggaggacgacgacga 25 agaggacgaggatgatgaggatgccgaagatgacgatggagacgagaacgatggcctggacaagaacaacgaggtggccg aggatgatgagaatgtcgtcgccccagagattgatcgcattaatgagaatatcaacaagactcgtgtagatggtctgcaaacat tgcatgcaatctgctttggcgcccaaggcaagaacaatgtggtcaagaagaacttgcgatcctttgccggtttcgagtttgccaaggatt cagcgg ag tacaacaaaaag ctgg ag gccatcaaaaag gtggataataag ggcctgcg cagcatctgcg ag atccttaccc30 gggtgatgaggaggaggaggaggatgccgaggacgaggatctggatgaagatgaggaggacccgcccagtgaagaggaca agaagegeaagageggaaagtetageggeggegetggeagaggetetgeaegeaatteeaeeggaegteeaaggegegega aaatgatgactccgagtcgggctcagattacaatccttctgccaattccgactctgacggtggtcgtggtggtggtgctggtgcagc aggtegeaaagteceaageegeggtggaegeggtegteetgegegeaaaagtegeagaagaaactetgatteegaggaagaa 35 gaggaatcggaagtttccgatgccgatagtgatgtcccaaaacgtaaacgtggttccgtgggtaaacgtggacgaccggcagct cctgcgtcagctggacgaaggggtagaggacgaggtgcagcttcccgcaagcgtaaagattcagatagcgaagatgaggagg tatccgaggatgaagaggaggatgtctccgattttgccagcgatcaaagcgaagtatgtaaatttaatttaatatcgagcatttg gtgttttatcaagtatatgccaatttttcaggaggaacgtcccaaaaagagcaagaagcccattacgcctgcgaaaaatagcaaag ctaacaacaagtcaaaaccagctggaaaggccgatagtcgaacaaaatcaaagaaggaatcgtccgaggaagatgatgat 40 gtcgatgacaaagatgaatccgacgaggatgagccactaaccaaaaagggcaaacaggcattcccaacggatgaacaaatacg cggatatgtcaaagagattctggataaagccaatcttgaggagattacgatgaaaaccgtgtgcaaacaagtttatgcaaaatatcc actgatcccgcgtggccgaacaacggttacaatatggttgatctgctgttgcaacaatcagatatttggggagacgtaa

45. Human Homologue TBLASTN with ORF1: poor homology with DEK gene (D6S231E) (NM_003472.1)
Drosophila EST several including LD33301 (AA979048)

204

| | | z genome genomic segment z genome Complete gene candidate | AE003805 CG5935 - EG:EG0003.6 - novel with weak homology to |
|----|--------------------|---|---|
| 5 | | | DEK oncogene CG8648 - EG:EG0003.3 - novel XPG/ flap endonuclease-like, DNA |
| 10 | Human homologue of | f Complete gene candidate | repair? CG5935- 1e-17 4503249 ref[NP_003463.1 pD6S231E DEK gene >gi 544150 sp P35659 DEK_H |
| 15 | | • | UMAN DEK PROTEIN >gi 284375 CG8648- 4758356 |
| 20 | | | ref NP_004102.1 pFEN1 flap structure-specific endonuclease 1; MATURATION FACTOR 1 (MF1); DNase IV; RAD2_HUMAN(aa) |
| 25 | Putative function | CG5935: function unknown but putative DNA-binding protein predicted to be involved in chromosomal organisation. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can | |
| 30 | | mRNA CG8648: Novel XPG/ flap endonuc | lease-like, DNA repair protein |

Confirmation by RNAi Both show slight reduction of G1 peak

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206

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Each of the applications and patents mentioned above, and each document cited or referenced in each of the foregoing applications and patents, including during the prosecution of each of the foregoing applications and patents ("application cited documents") and any manufacturer's instructions or catalogues for any products cited or mentioned in each of the foregoing applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

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Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

PCT/GB01/01297

CLAIMS

WO 01/72774

- 1. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 70 or the complement thereof.
- 5 (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 70, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 70 or a fragment thereof.
- 10 (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 2. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 14 or the complement thereof.
- 15 (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 14, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 14 or a fragment thereof.
- 20 (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 3. A polynucleotide selected from:

- (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 15 to 19 or the complement thereof.
- (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 15 to 19, or a fragment thereof.
- 5 (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 15 to 19 or a fragment thereof.
 - (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
- 10 4. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 20 to 30 or the complement thereof.
 - (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 20 to 30, or a fragment thereof.
- 15 (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 20 to 30 or a fragment thereof.
 - (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
- 20 5. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 31 to 53 or the complement thereof.
 - (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 31 to 53, or a fragment thereof.

- (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 31 to 53 or a fragment thereof.
- (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 6. A polynucleotide selected from:

- (a) polynucleotides comprising any one of the nucleotide sequences set out in54 to 70 or the complement thereof.
- (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in 54 to 70, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 54 to 70 or a fragment thereof.
- (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 7. A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a polynucleotide according to any of Claims 1 to 6.
- 8. A polypeptide which comprises any one of the amino acid sequences set out in Examples 1 to 70 or in any of Examples 1 to 14, Examples 15 to 19, Examples 20 to 30,
 20 Examples 31 to 53 and Examples 54 to 70, or a homologue, variant, derivative or fragment thereof.
 - 9. A polynucleotide encoding a polypeptide according to Claim 8.
 - 10. A vector comprising a polynucleotide according to any of Claims 1 to 7 and 9.

11. An expression vector comprising a polynucleotide according to any of Claims 1 to 7 and 9 operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.

210

- 12. An antibody capable of binding a polypeptide according to Claim 8.
- 5 13. A method for detecting the presence or absence of a polynucleotide according to any of Claims 1 to 7 and 9 in a biological sample which comprises:
 - (a) bringing the biological sample containing DNA or RNA into contact with a probe according to Claim 9 under hybridising conditions; and
- (b) detecting any duplex formed between the probe and nucleic acid in the sample.
 - 14. A method for detecting a polypeptide according to Claim 8 present in a biological sample which comprises:
 - (a) providing an antibody according to Claim 12;
 - (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
 - (c) determining whether antibody-antigen complex comprising said antibody is formed.
 - 15. A polynucleotide according to according to any of Claims 1 to 7 and 9 for use in therapy.
- 20 16. A polypeptide according to Claim 8 for use in therapy.

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17. An antibody according to Claim 12 for use in therapy.

- 18. A method of treating a tumour or a patient suffering from a proliferative disease comprising administering to a patient in need of treatment an effective amount of a polynucleotide according to any of Claims 1 to 7 and 9.
- 19. A method of treating a tumour or a patient suffering from a proliferative disease,
 5 comprising administering to a patient in need of treatment an effective amount of a polypeptide according to Claim 8.
 - 20. A method of treating a tumour or a patient suffering from a proliferative disease, comprising administering to a patient in need of treatment an effective amount of an antibody according to Claim 12 to a patient.
- 10 21. Use of a polypeptide according to Claim 8 in a method of identifying a substance capable of affecting the function of the corresponding gene.
 - 22. Use of a polypeptide according to Claim 8 in an assay for identifying a substance capable of inhibiting the cell division cycle.
- 23. Use as claimed in Claim 22, in which the substance is capable of inhibiting mitosis and/or meiosis.
 - 24. A method for identifying a substance capable of binding to a polypeptide according to Claim 8, which method comprises incubating the polypeptide with a candidate substance under suitable conditions and determining whether the substance binds to the polypeptide.
- 20 25. A method for identifying a substance capable of modulating the function of a polypeptide according to Claim 8 or a polypeptide encoded by a polynucleotide according to any of Claims 1 to 7 and 9, the method comprising the steps of: incubating the polypeptide with a candidate substance and determining whether activity of the polypeptide is thereby modulated.

- 26. A substance identified by a method or assay according to any of Claims 21 to 25.
- 27. Use of a substance according to Claim 26 in a method of inhibiting the function of a polypeptide.
- 28. Use of a substance according to Claim 26 in a method of regulating a cell division5 cycle function.